

1972

# Oxidation-Reduction Reactions of Sulfate and Sulfide in Flooded and Nonflooded Soil.

Robert Mclure Engler

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OXIDATION-REDUCTION REACTIONS OF SULFATE AND  
SULFIDE IN FLOODED AND NONFLOODED SOIL.

The Louisiana State University and Agricultural  
and Mechanical College, Ph.D., 1972  
Agronomy

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**OXIDATION-REDUCTION REACTIONS OF SULFATE  
AND SULFIDE IN FLOODED AND  
NONFLOODED SOIL**

**A Dissertation**

**Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy**

**in**

**The Department of Agronomy**

**by**

**Robert McLure Engler**

**B.S., Louisiana State University, 1967**

**M.S., Louisiana State University, 1969**

**August, 1972**

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## ACKNOWLEDGEMENT

The writer wishes to express his sincere appreciation to Dr. W. H. Patrick, Jr., Professor of Agronomy, Louisiana State University for his assistance and guidance during the course of this investigation.

The author is indebted to his associates for their assistance and suggestions throughout this study and to Dr. W. H. Willis, Head of the Agronomy Department, who was instrumental in making this study possible.

This research was supported in part by National Science Foundation Grant GB-8653 (General Ecology Section): due acknowledgement is made to this agency and to all other persons who in some way contributed to this investigation.

The writer wishes to express his deep gratitude to his wife Pat, whose patience, encouragement and ability as a typist has been an inspiration and made this work possible.

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## ABSTRACT

Studies were made on sulfate and sulfide transformations in a Crowley silt loam incubated under flooded and nonflooded conditions. A comparison of sulfide formation from an inorganic sulfur source ( $\text{Na}_2\text{SO}_4$ ) with an organic sulfur source (ground rice straw) was made. Approximately 18% of the added organic sulfur was recovered after 50 days of incubation. In contrast, about 90% of the added inorganic sulfur was recovered after 11 days of incubation.

The formation of sulfide from  $\text{MnSO}_4$ ,  $\text{FeSO}_4$ ,  $\text{CuSO}_4$ ,  $\text{AgSO}_4$ ,  $\text{BaSO}_4$ ,  $\text{CaSO}_4$ ,  $\text{PbSO}_4$ ,  $\text{MgSO}_4$ ,  $\text{Na}_2\text{SO}_4$ , and elemental sulfur added to a flooded soil was investigated. Copper and silver sulfate appeared to be toxic and no sulfide was produced. Approximately 4 ppm sulfide was recovered from barium sulfate. About 28% of the added elemental sulfur was recovered as sulfide. The reduction of other sulfate sources resulted in about 65 to 80% recovery of the added sulfur as sulfide.

The oxidizing effects of oxygen, ferric oxide, potassium nitrate, manganese dioxide, ferricitrophosphate, and ferric phosphate on the sulfide formation of a flooded soil were determined. The oxidants were mixed with the soil prior to flooding. The soluble oxidants (potassium nitrate and ferricitrophosphate)

retarded initial sulfide formation, while the more insoluble oxidants (manganese dioxide, ferric phosphate, and ferric oxide) had little effect on the initiation of sulfide production. However, ferric phosphate and manganese dioxide were effective in reducing the maximum rate of sulfide formation. In contrast, the soluble oxidants had no appreciable effect on the maximum rate of sulfide formation in the flooded soils. The oxygen treatment was only slightly effective in delaying the initiation and reducing the maximum rate of sulfide formation.

Oxygen, potassium nitrate, manganese dioxide, ferricitrophosphate, and ferric phosphate were added to a reduced, flooded soil in which sulfides had accumulated. The oxygen treatment was the most effective sulfide oxidant. The soluble oxidants (potassium nitrate and ferricitrophosphate) were less effective in oxidizing sulfides. In contrast, the insoluble oxidants were the least effective sulfide oxidants.

Manganous sulfide, ferrous sulfide, zinc sulfide, copper sulfide, and mercuric sulfide were mixed with the soil and were incubated under a flooded and non-flooded ( $1/3$  bar moisture tension) conditions. The oxidation and stability of the heavy metal sulfides were determined. The metal sulfides were relatively

stable in flooded soil. However, in the aerobic soil, the oxidation of the metal sulfide was relatively dependent on the solubility of the salt. The stability of the metal sulfide decreased with an increase in solubility of the salt.

Additional studies on mercury sulfide and mercury transformation under alternate nonflooded and flooded conditions were conducted. Mercury sulfide was relatively stable under anaerobic conditions. Soluble mercury ( $\text{HgCl}_2$ ) added to the flooded soil was rapidly rendered insoluble and was unextractable.

The oxidative effects of the growing rice root on heavy metal sulfides ( $\text{MnS}$ ,  $\text{FeS}$ ,  $\text{ZnS}$ ,  $\text{CuS}$ , and  $\text{HgS}$ ) placed in the root zone were studied. The oxidation of the metal sulfide was directly related to the solubility of the salt. The stability of the sulfide increased with a decrease in the solubility of the metal sulfide.



## INTRODUCTION

Studies as early as 1936 have shown the deleterious effect of  $H_2S$  on the rice plant (Oryza sativa). Several diseases of the rice plant have been shown to be associated with small concentrations of  $H_2S$  in paddy fields. Some of these diseases are: Akiochi disease of rice in Japan, Bruzone disease in Hungary, Straighthead disease of rice in the United States, and certain maladies of citrus grown on poorly drained soils of Florida. Several types of injury due to  $H_2S$  have been shown and may vary from complete necrosis of the rice root to decrease root enzyme activity and ion uptake by the plant.

A few methods of alleviating sulfide toxicity have been demonstrated, however, the basic principle of detoxifying soil sulfide was to create a less reduced soil system in which the reduction of sulfate would not occur. This was accomplished by the addition of highly oxidized compounds such as manganese dioxide and potassium nitrate to the paddy soil. Due to the relatively sequential reduction of the oxidized soil components, the soil redox system was "buffered" at a point where sulfate reduction would not occur.

The capability of  $H_2S$  to form very insoluble salts with heavy metals has been basically overlooked. The formation of  $MnS$  and  $FeS$  to remove sulfide from a flooded

system have been previously studied, but the formation of the very insoluble sulfide salts of zinc, copper, and mercury in flooded systems have not been clearly defined. Zinc and copper usually occur in small amounts in the soil and are required nutrient elements for plants. The formation of very insoluble and unavailable  $ZnS$  and  $CuS$  may be an important limiting factor in the availability of these metals for utilization by aquatic plants. On the other hand, the formation of insoluble and stable mercury sulfide may render harmless and unavailable an otherwise extremely toxic material.

The uptake of plant nutrients and respiration of the rice root demands the presence of oxygen in the zone of soil adjacent to the rice root. However, oxygen is relatively unavailable from the anaerobic paddy soil. Consequently, rice as well as other aquatic plants have the capability to supply the root zone with oxygen transported from the aerial parts to the root tips. Thus, a relatively oxidized area is formed in a zone of soil immediately around the rice root. Establishment of this relatively oxidized zone is only a portion of the multifaceted biochemistry of the paddy soil. With certainty, sulfide toxicity as well as the insoluble nature and stability of heavy metal sulfides are affected by this oxidized zone of soil adjacent to the rice root.

The overall objective of this research was to gain further insight into the oxidation-reduction reactions of sulfate and sulfide compounds in oxygen-deficient and aerobic soils. The specific objectives of this research were: (1) to compare sulfide formation from an organic sulfur source with that of an inorganic source of sulfur, (2) to contrast various inorganic sulfur compounds as a sulfide source, (3) to study sulfide production as affected by various oxidants added to a soil prior to submerging, (4) to evaluate sulfide accumulation as affected by various oxidants added to a reduced submerged soil after sulfide formation, (5) to study heavy metal sulfide reactions in aerobic and anaerobic soils, (6) to study reactions of mercury in aerobic and anaerobic soils, and (7) to determine the effect of the rice plant on metal sulfides in a submerged soil.

## REVIEW OF LITERATURE

### A. Sulfide Accumulation in Submerged Soils

When an aerobic, oxidized soil is submerged under water, the dynamic soil microbial population changes from a system where oxidative reactions are significant to a system where reduction of oxidized species is dominant. In anaerobiosis, the accumulation of highly reduced, volatile, and toxic compounds is common. One such transformation is the microbial reduction of the oxidized forms of soil sulfur. The end product, an important metabolic product of this anaerobic reductive process, is hydrogen sulfide. The predominant oxidized form of sulfur to be reduced in an anaerobic soil is sulfate sulfur. The major microorganisms involved in the reduction of sulfate are bacteria of the genus Desulfovibrio (Alexander, 1961).

Sulfides in Louisiana soils have been found to vary from a low of 0.2 ppm total sulfide for a submergence time of short duration (Rodriguez, Jordon, and Hollis, 1965) to a high of 10 to 45 ppm at the end of the growing season. Sturgis (1936) reported a sulfide content of 29.9 ppm in pot experiments using a Sharkey clay loam. The soil was continuously flooded for three weeks. Connell and Patrick (1969) found as much as 40 ppm total

sulfides in a flooded Louisiana paddy soil (Crowley silt loam). In other areas, total sulfide levels of submerged soils have been reported to increase to 150 ppm (Alexander, 1961) and in some cases to be as high as 2,000 ppm (Harter and McLean, 1965). It was determined by Ogata and Bower (1965) that total sulfide accumulation increased with time. They also demonstrated a positive correlation between the organic matter content of a given soil and sulfide formation.

Reports of the occurrence of free sulfide (noncomplexed or absorbed  $H_2S$ ) seem to be very inconsistent. In peaty rice soils of Japan, Takijima, and Mitsuhiro (1958) demonstrated that there was vigorous evolution of gases from the soil but that the free  $H_2S$  content was insignificant. Research by Bloomfield (1963) showed that in laboratory experiments, as much as half of the soil sulfate could be lost as volatile  $H_2S$ . Connell and Patrick (1969) found that in Louisiana soils almost no sulfide existed as  $H_2S$  if excess iron was present. However, a Texas soil (Hockley fine sandy loam) used in their study produced 7 ppm free  $H_2S$ . Hollis (1967), using theoretical values, showed that free sulfide levels in Louisiana paddy soils may be found to be in levels toxic to the rice plant.

## 1. Biochemistry of Soil Sulfide

When an aerated soil or sediment is submerged under water, several dynamic biochemical systems will predominate due to the consumption of soil oxygen by soil microorganisms. The need for electron acceptors by facultative anaerobic and obligate anaerobic organisms creates a systematic sequence of oxidation-reduction systems. The sequence of these systems may be predicted thermodynamically and have been demonstrated experimentally by several researchers (Ponnamperuma, 1955; Ponnamperuma, 1964; Redman and Patrick, 1965; Yaun and Ponnamperuma, 1966; Patrick and Mahapatra, 1968). The reduction of oxygen ( $O_2 - H_2O$  system), nitrate ( $NO_3^- - N_2$  system), the higher oxides of manganese ( $Mn^{+4} - Mn^{+2}$  system), hydrated ferric oxide ( $Fe^{+3} - Fe^{+2}$  system), and sulfate ( $SO_4^{-2} - S^=$  system) will occur in a more or less sequential fashion. The major controlling factors related to these reduction processes in submerged soils are microbial population, organic matter content, oxidation-reduction potential, and pH.

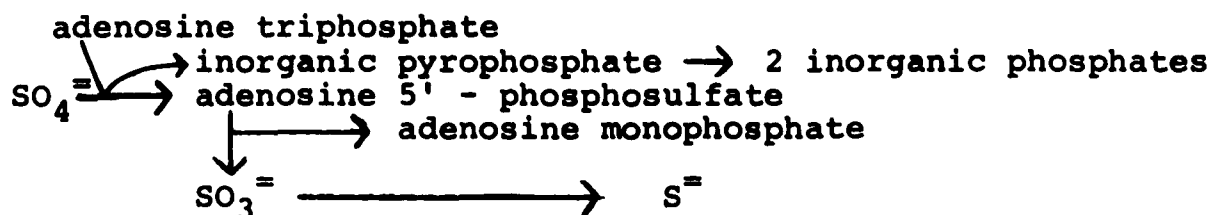
### a. Sulfate-Reducing Microorganisms

Sulfate reduction as described by Starkey (1950) is carried out primarily by a unique group of bacteria known collectively as sulfate-reducers. Sulfate reducing organisms can be placed into two distinct groups. Peck (1962) describes one group as those organisms that

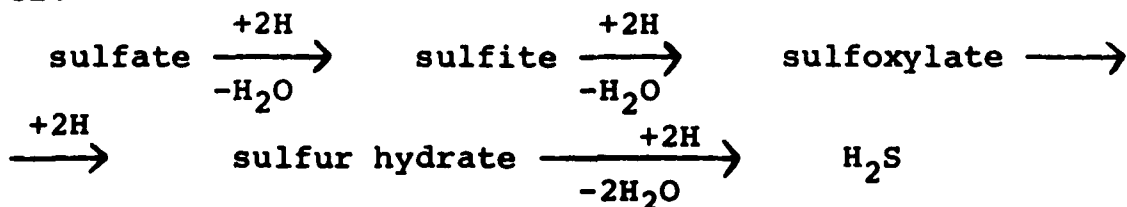
reduce sulfate but do not produce detectable amounts of sulfide and are called "assimilatory sulfate-reducers". The remaining class of sulfate-reducers has been described by Postgate (1959, 1965) as organisms whose major energy-yielding reactions are related to a reduction of sulfate to the sulfide ion and are called the "dissimilatory sulfate-reducers". These are usually referred to as the true sulfate-reducers.

Sulfate-reducers which predominate in soil are bacteria of the genus Desulfovibrio (Alexander, 1961). These organisms are known to reduce sulfate, sulfite, thiosulfate, and elemental sulfur to sulfide. Desulfotomaculum has also been shown to reduce sulfate to sulfide (Trudinger, 1969). Butlin, Adams, and Thomas (1949) have noted that Desulfovibrio desulfuricans appears to be the most widely distributed sulfate-reducers in nature. Clostridium nigrificans has been reported to reduce sulfate at elevated temperatures (Campbell, Frank, and Hall, 1956). Bacillus megaterium has been reported by Broomfield (1953) to reduce sulfate to  $H_2S$  but only under certain controlled conditions not common to a normal soil environment.

A pathway for sulfate reduction has been elucidated (Alexander, 1961; Peck, 1962; Trudinger, 1969) and follows:



or:



#### b. Effect of Organic Matter

A study of Ogata and Bower (1965) has shown that there was a positive relation between the accumulation of sulfide and the soil organic matter content. Organic matter was found to be the prevalent regulatory mechanism for sulfide accumulation as long as the sulfate content of the soil was not limiting. Rice grown in Japan on poorly drained soils containing significant amounts of organic matter exhibited symptoms resembling those caused by  $\text{H}_2\text{S}$  (Baba and Harrada, 1954). Experiments conducted by Yamane and Sata (1961) using muck soils demonstrated that decomposable organic matter acted as a substrate for sulfate reducing organisms.

Bloomfield (1969) reported that the source of organic matter had a marked effect on sulfide evolution. Considerably more free  $\text{H}_2\text{S}$  was evolved when lucerne was used as an energy source as compared to rice straw.



In peat, muck or mineral soils which receive a large amount of organic matter considerable soil sulfur may be in the organic form. Sulfhydryl containing proteins and amino acids yield  $H_2S$  upon decomposition (Alexander, 1961). In an aerated environment, the sulfide sulfur is mineralized to the sulfate form and could then be reduced to  $H_2S$  if the environment became anaerobic (Millar, 1955). In an anaerobic environment, the organic sulfide may not be mineralized and would remain in the reduced state and contribute to the accumulation of total soil sulfide. Starkey and Wight (1945) determined that sulfide formation from organic sulfur sources was secondary when compared to the reduction of sulfates.

c. Effect of Oxidation-Reduction Potential

Sulfate reducing microorganisms are classed as obligate anaerobes and are only active in the absence of free oxygen. A low (negative) oxidation-reduction potential is also a criterion for the reduction of sulfate to sulfide (Starkey and Wight, 1945; Starkey, 1950; Postgate, 1959; Harter and McLean, 1965; Connell and Patrick, 1968; 1969). Harter and McLean (1965) determined that sulfate-reducing microorganisms functioned best at Eh potentials of -75 millivolts or less and that at oxidation-reduction potentials below -75 millivolts sulfate-reducers were extremely active. Their work

was based on the fact that the sulfide content of their soil increased at a fast rate at potentials less than -75 millivolts. Connell and Patrick (1968, 1969) demonstrated in an incubated soil that the critical oxidation-reduction potential for the inception of sulfate reduction was about -150 millivolts. Their work was accomplished utilizing an automated system to control the oxidation-reduction potential at a desired Eh level. In an earlier study, Postgate (1959) proposed that the soil Eh should be about -200 millivolts in order to stimulate the microbial reduction of sulfate to sulfide. The concentration of soil sulfate was found to have a significant effect on the activity of sulfate-reducers (Starkey and Wight, 1945) and their activity was reported to be important in lowering the oxidation-reduction potential. In a later experiment, Starkey (1950) concluded that at the inception of microbial (sulfate-reducers) growth, intense anaerobic conditions increased due to the fact that the  $H_2S$  produced was a strong reducing agent. Postgate (1959) demonstrated that the oxidation-reduction potential could be lowered to -200 millivolts by the addition of a solution containing 15 ppm of  $H_2S$ . The utilization of oxidation-reduction potential measurements is of great value in characterizing an anaerobic soil environment for the possible production of  $H_2S$ .

d. Effect of pH

Soil pH is another important factor controlling the accumulation of  $H_2S$  in a flooded soil. Alexander (1961) found that the acid pH limit for the microbial evolution of  $H_2S$  was pH 5.5. From these data he postulated that this was the reason for the absence of sulfate reduction in very acid soils. A pH of 7.0 was reported to be optimum for sulfide production (Starkey and Wight, 1945) and the active range of sulfate reduction was pH 5.5 to 8.5. Connell and Patrick (1969) reported that the pH range for sulfide accumulation in a Crowley silt loam to be between pH 6.0 and 9.5. The maximum sulfide accumulation was found to be at pH 6.8. Hollis (1967) calculated theoretical  $H_2S$  concentrations under Louisiana rice field conditions and reported theoretical sulfide concentrations of 100, 16.7, 1.7, 0.17 and 0.017 ppm at pH values of 5.0, 5.5, 6.0, 6.5 and 7.0, respectively. Sulfide production in marine sediments was reported to occur from pH 6.4 to 9.5 and from pH 4.2 to 10.4 in waterlogged soils (Zobell, 1958). Bloomfield (1969) reported that more free  $H_2S$  was generated under alkaline conditions but he added that iron was more immobile under alkaline conditions and that the iron system governed the distribution of sulfide in a reduced soil.

## 2. Sulfide Toxicity

The toxicity of  $H_2S$  to soil microorganisms and higher plants has been well documented. Therefore, the reduction of sulfate to sulfide in rice fields where organic matter, sulfate fertilizers, and anaerobic conditions are prevalent, can be of great importance to the rice producer. An early study (Sturgis, 1936) into the toxic substances produced under anaerobiosis gave the first published evidence of the harmful effects of  $H_2S$ . Hollis (1967), in a comprehensive review of the Japanese literature concerning sulfide toxicity of the rice plant, reported that Mitsui and his co-workers exposed rice roots to a solution containing 0.07 ppm  $H_2S$  and caused wilting of 42% of the leaves at the end of 200 hours. They also demonstrated that the same amount of wilting was evident at the end of 48 hours when the  $H_2S$  concentration was 2 ppm. They demonstrated that passing  $H_2S$  through culture solutions at a rate of 2 bubbles per second for 30 minutes caused a marked reduction in ion accumulation, water absorption, and protein synthesis.

In a study by Vamos (1958), it was shown that  $H_2S$  at pH 7 affected and could destroy the roots of the rice plant and at pH 8 the plant roots absorbed the  $SH^-$  ion which resulted in a browning of the tissue. Vamos also reported  $H_2S$  to be the causative factor of the disease

"bruzone or akiochi", a significant disease of the rice plant in Hungary and Japan.

It is well documented that  $H_2S$  is an inhibitor of the enzymes of aerobic respiration (James, 1953a; 1953b; Dixon and Webb, 1964). Paul (1951), Nicholls and Schonbaum (1963), and Paul (1963) have reported on the inhibitive effects of  $H_2S$  on the iron-containing enzymes. Mitsui and Kumazawa (1964) in their studies of the nutrient uptake by the rice plant have postulated that the reduced uptake of certain nutrients was associated with the enzymatic reactions affected by  $H_2S$  toxicity. Sulfide toxicity to the iron containing enzymes cytochrome oxidase, catalase, and peroxidase has also been reported by Ponnampersuma (1955).

In a study on temporarily flooded citrus areas of Florida, Ford (1965) observed that sulfides linked with oxygen deficiency was a probable cause in the death of citrus roots. Ford (1968) in later studies found that rapid destruction of feeder roots occurred when the citrus areas were subjected to flooding. He associated this toxicity to the production of sulfides rather than to the direct effect of oxygen deficiency.

Okajima and Takagi (1953) studied the effect of  $H_2S$  on the absorption of nutrients and observed the degree of inhibition of nutrient uptake to be in the order of  $P_2O_5 > K_2O > NH_3 > NO_3 = H_2O$ . Okajima and

Takagi (1955) further elucidated the harmful effects of  $H_2S$  and found that in the presence of  $H_2S$  rice yield was reduced, translocation of inorganic nutrients was inhibited, and the formation of carbohydrate was limited. Okajima and Takagi (1956) also reported that the presence of  $H_2S$  in the root zone of a culture media inhibited the translocation of foliar applied radioactive  $^{32}P$ .

### 3. Source of Sulfide

Takai, Koyama, and Kamura (1956, 1964) reported that sulfides were derived from sulfates present when anaerobic conditions developed or from sulfates applied after flooding. The reduction of sulfate is especially important because a considerable amount of sulfate in the form of ammonium sulfate is applied to rice soils each year. Jordan and Emsinger (1958) reported that most of the inorganic sulfur present in an aerated soil was present in the sulfate form. A thorough study of sulfur sources other than sulfate for microbial reduction was carried out by Postgate (1951). He reported that D. desulfuricans utilized sulfite, thiosulfate, tetrathionate, metabisulfite, dithionite, and to a small degree elemental sulfur in the place of sulfate for growth. He also found that the organism was unable to grow with or reduce dithionate, perdisulfate, formaldehydesulphorylate, sulfamate, benzenesulfonate,

methanesulfonate, beta-hydroxyethane-sulfonate, sodium ethylsulfate, dimethylsulfone, or cystine.

Under certain conditions, the prevalent form of soil sulfur may be in the organic form and the decomposition of this organic matter can lead to the accumulation of sulfides. Millar (1955) reported that under aerated conditions, organic bound sulfur could be oxidized to the sulfate form. However, Starkey and Wight (1945) assume that sulfide accumulation from an organic sulfur source is of little consequence as compared to sulfate reduction. Postgate's (1951) work exhibited almost no sulfide evolution from the organic sulfur sources under study.

#### 4. Forms and Distribution of Soil Sulfide

Microbial reduction of sulfur results in an evolution of  $H_2S$  that was limited only by the several factors previously reviewed. However, it is highly unlikely that the sulfide remains in that form. According to Motomura (1969) hydrogen sulfide is under a dynamic physiochemical equilibrium with respect to soil conditions. Komarov and Volneiko (1964) reported that the specific nature of the soil clay fraction was a significant influence on  $H_2S$  accumulation.

Research by Harter and McLean (1965) demonstrated that in a soil producing high amounts of sulfide almost all of the sulfide was precipitated as ferrous sulfide.

The soil under study was high in extractable ferrous iron when flooded and the extractable ferrous iron decreased with an increase in sulfide. On the contrary, Bloomfield (1963) reported as much as half the sulfate in the soil was sometimes lost as  $H_2S$ , although he concluded that it was possible that the release of  $H_2S$  was due to too great an acidity rather than from a deficiency of iron.

Connell and Patrick (1969) showed that there existed as much as 7 ppm "free"  $H_2S$  in a Texas soil (Hockley fine sandy loam). Hollis (1967) postulated that the presence of "free"  $H_2S$  in the soil solution could be significant.

Generally speaking, other sulfide compounds should be present in a flooded soil. Hydrogen sulfide readily forms sulfide salts with heavy metals present in solution with the  $H_2S$ . Consequently, soil solutions containing manganese, copper, zinc, and mercury would contain the sulfide salts of these metals when  $H_2S$  was introduced to the system. Thermodynamically the formation of these salts should generally follow the pattern set by the formation of ferrous sulfide.

Little information can be found on the sulfide distribution throughout a soil profile. But one can predict the sulfide distribution to some extent by determining the sulfate distribution in a soil profile.



Whittig and Janitzsky (1963) reported the sulfide content to a depth of 40 inches of soil, but gave the total sulfide content to the depth rather than a distribution within the profile. The formation of  $H_2S$  was reported by Vamos (1958) to be in the upper 3 to 4 cm layer of soil.

Sulfate concentrations to southern soil profiles have been reported by Jordan (1964) to be less than 3 ppm in the 0 to 6 inch horizon, and 6 to 187 ppm in the 18 to 24 inch horizon.

B. Effect of Oxidizing Compounds on Sulfide Accumulation in Submerged Soils

Sulfide, one of the most reduced components in an anerobic soil is also one of the most reactive with oxygen and other oxidized compounds. Prior to the evolution of sulfide there must be a total absence of oxygen or other oxidized forms such as nitrate, nitrite, or manganese. In theory, nitrate or manganese contained in a soil upon submergence would inhibit or depress the production of  $Fe^{++}$ ,  $H_2S$ , and the organic products of anaerobic bacterial metabolism which operate at low oxidation-reduction potentials. This is generally due to the fact that the nitrate, nitrite, and manganic oxidation-reduction systems have standard oxidation-reduction potentials which are considerably

higher than those of iron, sulfide, and organic systems likely to operate in flooded soils (Ponnamperuma, 1955; Ponnamperuma and Castro, 1964). Another significant reason for the delaying action is the fact that a wide variety of bacteria utilize  $\text{NO}_3^-$  (Allen and van Niel, 1952; Verhoeven, 1956; Gilmour, Damsky and Bollen, 1958) and  $\text{MnO}_2$  (Mann and Quastel, 1946; Hochster and Quastel, 1952) prior to  $\text{SO}_4^{=}$  as electron acceptors in their respiration.

Soil reduction appears to follow a more or less sequential pattern with oxygen being reduced first followed by nitrate, manganic manganese, ferric iron, intermediate breakdown products of organic matter, and sulfate (Ponnamperuma, 1955; Ponnamperuma, 1964; Patrick and Mahapatra, 1968; Turner and Patrick, 1968). Oxidation of these components might follow a more or less reverse sequence.

#### 1. Effect of Oxygen

Molecular oxygen is extremely reactive with reduced compounds, especially the reduced forms of sulfur. Movement of molecular oxygen into a reduced soil profile would result in a complete oxidation of the reduced soil components. A study by Connell (1966) showed that a soil containing 120 ppm total sulfides when exposed to air and agitated decreased to 5 ppm total sulfide after 2 hours. He also found that in

an incubated study the addition of oxygen had a delaying effect on sulfate reduction.

Microbial sulfate reducers are classified by Alexander (1961) as obligate anaerobes and states that the microorganisms are inactive in the presence of molecular oxygen.

## 2. Effect of the Oxides of Nitrogen

The stabilization of the oxidation-reduction potential at 200 to 400 millivolts at pH 7, and the absence of significant concentrations of typical reduction products in anaerobic systems containing nitrate, has been reported by several researchers (Pearsall, 1938; Mortimer, 1941, 1942; Ponnampereuma, 1955; Vamos, 1958; Ponnampereuma and Castro, 1964; Ponnampereuma, 1964; Yaun and Ponnampereuma, 1966; Turner and Patrick, 1968).

Nitrate reduction follows closely the reduction of oxygen when a soil is flooded and as long as sufficient nitrate is present in the soil solution the next reduction reaction will proceed slowly until all of the nitrate is reduced (Turner and Patrick, 1968). In their study, as long as nitrate was present, the oxidation-reduction potential was sufficiently high to render the sulfate-reducers inactive.

A proposed method of controlling  $H_2S$  was given by Vamos (1958). He found that when nitrate was added to

a flooded soil system, the microbially produced atomic hydrogen was used to reduce the nitrate and in turn, could not be used by the sulfate-reducers as an energy source. Therefore, the addition of nitrate prevented only  $H_2S$  evolution. Similar results were achieved in field experiments utilizing ammonium nitrate mixed with powdered lime. Also, Ponnamperuma (1955) demonstrated that applications of nitrate depressed the concentration of ferrous iron and organic reduction products and markedly improved the growth of rice in a flooded soil.

Yaun and Ponnamperuma (1966) reported that nitrate at 0.4% by weight of soil retarded reduction, but was itself toxic on acid soils. Connell (1966) found that as long as nitrate was present in a submerged soil no sulfate was reduced to sulfide.

### 3. Effect of the Oxides of Manganese

It was observed by Ponnamperuma, Yaun, and Nhung (1965) that soils, naturally high in  $MnO_2$ , underwent slow reduction upon flooding. Turner and Patrick's (1968) data demonstrated a buffering effect by the oxides of manganese upon the oxidation-reduction potential of a flooded soil. They reported the oxidation-reduction potential to be greater than zero until manganous accumulation had reached a maximum. This potential has been reported to be sufficiently high to

inhibit sulfate reduction. Ponnamperuma, Yaun, and Nhung (1965) demonstrated the ability of manganese dioxide to correct toxic conditions associated with highly reduced soil. Manganese dioxide was added at 0.4% by weight of soil.

It has also been shown, as for nitrate, that manganese dioxide can act as the terminal electron acceptor of the microbial respiratory system (Hochster and Quastel, 1952; Mann and Quastel, 1946).

#### 4. Effect of the Oxides of Iron

Shortly after a soil is submerged under water, the microbiological reductive processes follow partially sequential steps. Intermediate in the reduction process is the reduction of the oxides of iron (Ponnamperuma, 1964; Turner and Patrick, 1968). The potential at which iron reduction initiates has been reported to be about +150 millivolts. This oxidation-reduction potential is considered sufficiently high enough to retard sulfate reduction.

Oxygen and nitrate are very important in retarding reduction, but are usually present in such low amounts that they are soon completely reduced and retard reduction for only a short while. On the other hand, the oxides of iron are present in much higher quantities and, in general, have a large reserve of solid phase oxidizable matter. The iron must be almost completely

reduced before intense reduction can set in and prolonged submergence is usually necessary before all of the hydrated ferric oxide is reduced. Turner and Patrick (1968) showed a buffering effect on the oxidation-reduction system as the oxidized iron was being reduced.

Japanese soils high in iron oxides are reported to produce very little free  $H_2S$  and the soluble sulfide content is considered insignificant (Takijima, Shiojima, and Konno, 1962). Harter and McLean (1965) found that any free  $H_2S$  produced in a flooded soil was precipitated as ferrous sulfide and demonstrated the fact that a decrease in the amount of ferrous iron occurred concurrently with an increase in the sulfide content. Ponnampetuma (1964) states that in soils abundant in iron, the presence of iron in the soil solution keeps the concentration of  $H_2S$  below  $1 \times 10^{-8}$  M and that  $H_2S$  toxicity is possible only in iron deficient soils. Alexander (1961) reported that total sulfide levels of submerged soils are reflected mainly as iron sulfide. Bloomfield (1969) reported that a very reactive synthetic source of Fe ( $Fe_2O_3$ ) in a slight excess of Fe over the  $SO_4^{-2}$  equivalent sufficed to prevent free sulfide accumulation.

Other researchers have pointed out that the soil iron system was relatively insignificant in retarding sulfide production in a flooded soil. Motomura (1969)

found only one form of ferrous iron producing exchangeable cations in submerged soils, which severely reduced the postulated effect of the large reserves of soil iron. Roberts (1947) and Broomfield (1954) theorized that the presence of  $\text{Fe}(\text{OH})_3$  has little appreciable effect on the reduction of a soil despite its presence in large amounts in the soil. Yamane and Sata (1961) reported that the additions of iron did not affect the evolution of  $\text{H}_2\text{S}$  from the soil. They believed that the iron was inactivated by soil organic matter into a complex formation. Bloomfield (1963) reported evolution of  $\text{H}_2\text{S}$  from a flooded soil even though soluble ferrous iron compounds and excess ferric oxide were present. He also stated that  $\text{H}_2\text{S}$  evolution could have been due to acidity.

#### C. Effect of Sulfide on the Heavy Metals in Submerged Soils

There are many reports in the literature on the oxidation-reduction transformations undergone by manganese and iron (Mortimer, 1941; Mann and Quastel, 1946; Roberts, 1947; Hochester and Quastel, 1952; Broomfield, 1954; Ponnampereuma, 1955; Alexander, 1961; Bloomfield, 1963; Ponnampereuma, 1964; Harter and McLean, 1965; Redman and Patrick, 1965; Hollis, 1967; Turner and Patrick, 1968; Mortomura, 1969). On the other hand, the evidence

of transformations undergone by zinc, copper and mercury is scarce.

Soils containing oxides of manganese and iron contain very little soluble Fe and Mn species in the oxidized state. However, when a soil is submerged, soluble iron and manganese forms accumulate at a rate limited only by the concentration of the oxidized forms, the activity of the reduction processes taking place, and the precipitation reactions involving ferrous and manganous ions. Turner and Patrick (1968) reported that the exchangeable manganese in a Crowley silt loam increased from about 20 ppm to 280 ppm after 7 days of submergence. They reported that the exchangeable ferrous iron increased from about 5 ppm to 360 ppm in a Sharkey silty clay after 7 days of submergence.

Zinc, a required micronutrient element for all living organisms, exists in most mineral soils in small quantities and any transformations undergone under flooded conditions would certainly affect its availability. Besides the metallic form, zinc exists only in one oxidation state and is not directly affected by the reduction processes. The zinc status would be affected by the formation of insoluble precipitates with other products of anerobiosis.

Stability diagrams of copper compounds at various Eh - pH levels show copper existing at various oxidation



states (Garrels and Christ, 1965). The oxidation-reduction status of a flooded soil would have a marked effect on copper solubility. The formation of insoluble salts with reduction products of other anaerobic reactions should closely regulate the concentration of soluble copper.

Mercury reactions in submerged environments have become very important in light of recent reports of mercury pollution in lakes, rivers, and bays. The oxidation-reduction and pH conditions in sediments that favor mercury transformations from insoluble to more soluble forms are little understood. For example, some investigators report that very toxic methylmercury is formed under anaerobic conditions (Wood, Kennedy, and Rosen, 1968; Jernelov, 1969; Jernelov and Jenson, 1969; Jernelov, 1970), while others indicate that oxygen is necessary for the reaction to occur (Wallace, Fulker-son, Shultz, and Lyon, 1971). Similar to iron, manganese and copper, mercury exists in more than one oxidation state and oxidation-reduction potentials may exist in nature that would transform mercury from one phase to another.

1. Complexing of Heavy Metals by Sulfide

Metal-sulfide complexes are quite common in nature and in some cases are the more important ore-form of a particular metal. Some common sulfide-metal complexes

existing as naturally-occurring ores are: pyrite ( $\text{FeS}_2$ ), sphalerite ( $\text{ZnS}$ ), cinnabar ( $\text{HgS}$ ), and covellite ( $\text{CuS}$ ) (Garrels and Christ, 1965). Manganese and copper will readily form sulfide salts as long as the Mn or Cu are the major cation species in solution and  $\text{S}^{=}$  is a major anion species (Krauskopf, 1967).

a. Iron Sulfide

Ferrous ion will stay in solution if the solution is reducing and slightly acidic, and if it is not admixed with other ions that form insoluble compounds. A great variety of ions may cause precipitation, the most common ones being carbonate, sulfide, and silicate. Berner (1970) states that once  $\text{H}_2\text{S}$  is formed in flooded sediments, it reacts with various iron containing minerals to form insoluble iron sulfide. He further states that the  $\text{FeS}$  (mackinawite) formed is soluble in hot concentrated  $\text{HCl}$ . In another study, Berner (1964) points out that iron sulfides are stable forms in flooded sediments under reducing conditions. Several authors (Ponnamperuma, 1955; Ponnamperuma, 1964; Ford, 1965; Harter and McLean, 1965; Connell and Patrick, 1968; Bloomfield, 1969) have postulated that the iron sulfide formation was important in regulating the amounts of toxic  $\text{H}_2\text{S}$  in the soil solution. This complex will also regulate the concentration of ferrous ions in solution.

The oxidation of iron sulfide is very important because most flooded rice soils go through a drying period where a complete oxidation of the system may occur. Hem (1960) showed that in a reduced system an increase in potential to 400 millivolts caused oxidation of ferrous sulfide with a corresponding redeposition of the iron as oxide or hydroxide. The sulfide was oxidized stepwise to the sulfate form. Krauskopf (1967) gives a possible mechanism of this reaction to be:  $2\text{FeS}_2 + 15/2 \text{O}_2 + 4\text{H}_2\text{O} \longrightarrow \text{Fe}_2\text{O}_3 + 4\text{SO}_4^{=2-} + 8\text{H}^+$ .

b. Manganese Sulfide

Krauskopf (1967) found that manganese reactions in reduced environments resembles very closely those of iron. In solutions with low oxidation-reduction potentials, manganese forms carbonates, sulfides, and silicates that are fairly insoluble in neutral or basic solutions. Manganese sulfide is also readily oxidized under aerated conditions to give very insoluble oxides of higher valence. Oxidation of manganous compounds requires higher potentials than does oxidation of ferrous compounds and manganese sulfide is more soluble than iron sulfide (Krauskopf, 1967). Connell and Patrick (1969) reported that reduced manganese was found to be less efficient than reduced iron in precipitating  $\text{H}_2\text{S}$ . The fact that  $\text{FeS}$  is more insoluble than  $\text{MnS}$  indicates that iron is a more effective

sulfide precipitating agent than manganese. When a flooded soil is drained however, oxidation of manganese and iron sulfide follow similar steps.

#### c. Zinc and Copper Sulfide

Little agronomic research has been done on zinc and copper reactions in flooded soils and sediments. Soluble zinc and copper are usually present in low concentrations in soil solutions. It has been demonstrated by geologists that zinc and copper readily form very insoluble sulfide salts when exposed to  $H_2S$  (Krauskopf, 1967). In addition, important ore deposits of zinc and copper are found as the sulfides sphaalerite ( $ZnS$ ) and covellite ( $CuS$ ) which are quite stable at high oxidation-reduction potentials. Although stable, zinc sulfide may undergo an oxidation reaction described by Krauskopf (1967) and was shown to be  $ZnS + 2O_2 + 2HCO_3^- \longrightarrow ZnCO_3 + H_2CO_3 + SO_4^{=}$ . The zinc is still in a relatively insoluble and stable form, but the sulfide has been oxidized to a readily "available" form. It can be postulated that the even more insoluble  $CuS$  will undergo a similar reaction but to a lesser degree.

#### d. Mercury Sulfide

Mercury sulfide is the least soluble ( $K_{sp} = 10^{-50}$ ) of the metal-sulfide salts (Garrels and Christ, 1965) and the formation of this salt should render both the mercury and the sulfide very stable. In a flooded

environment, mercury is generally present either as one of a variety of inorganic or humic complexes or as the relatively unionized and stable mercury sulfide (Geological Survey Staff, 1970). Under oxidizing conditions, however, mercuric sulfide is gradually converted to the more soluble mercuric sulfate (Wiklander, 1969).

Because of mercury's tendency to sorbe readily on a variety of silicate and organic materials, particulate matter suspended in water and bottom sediments of streams are more likely to contain higher concentrations of mercury than the water itself, regardless of the source. The best estimate is that suspended matter may contain from 5 to 15 times as much mercury as the water around it in areas of industrial pollution (Geological Survey Staff, 1970). Due to the dynamic nature of the oxidation-reduction systems present in flooded sediments and soils, mercury will certainly enter into many of these transformations. Fagerstrom and Jernelov (1970) demonstrated that highly soluble and toxic methylmercury was formed from pure mercuric sulfide in aerobic organic sediments although the process was considerably slower than when divalent mercury ( $\text{HgCl}_2$ ) was the mercury source. This suggests that the highly insoluble and stable  $\text{HgS}$  was rendered unstable in a dynamic microbiological environment.

D. Effect of the Rice Plant on Sulfide in Submerged Soils

The rice plant, similar to other aquatic plants, has exhibited the ability to move gaseous oxygen through the stem and to diffuse the oxygen into the soil immediately adjacent to the root hairs. Several workers (Alberda, 1953; Aomine, 1962; Armstrong, 1964; Mitsui, 1964; Ponnampetuma, 1964; Rodriguez, Jordan, and Hollis, 1965; Armstrong, 1967) have demonstrated the rice plant's ability to ward off some of the toxic products of soil reduction by the secretion of oxygen by the root hairs into the adjacent soil. Armstrong (1964) measured the oxygen diffusing from the roots of the rice plant to progress at a rate of  $12 \text{ g} \times 10^{-8} \text{ O}_2$  per  $\text{cm}^2$  root surface per minute. He suggested that the tolerance of some aquatic plants to toxic  $\text{H}_2\text{S}$  was due to the rapid diffusion of oxygen from the roots and subsequent oxidation of the sulfide. Armstrong (1967) postulated that soluble iron salts would be expected to act as sinks for oxygen diffused through the plant roots. Support for this hypothesis was provided by the thick covering of hydrated ferric oxide that was generally found around the roots of plants growing in reduced soils. Armstrong (1967) also suggested that the diffusion of oxygen from the roots in an anaerobic medium

may be necessary to maintain an external oxygen supply to the meristem as well as protection from soil toxins. In an article by Alberda (1953), it was shown that the rice plant demonstrated the movement of oxygen down the stem and its secretion from the roots. In a pot study, Rodriguez, Jordan, and Hollis (1965) measured approximately 4 ppm  $O_2$  in the soil water adjacent to the roots of rice plants grown in flooded oxygen deficient soil. A mechanism for the movement of oxygen through the root to the surrounding soil was theorized by Mitsui (1964). He postulated a glycolic acid pathway as the mechanism for movement of oxygen into the rice root tip and from the root tip into the soil. A review by Aomine (1962) reported on the obvious mottling of grey reduced areas of a flooded soil with red oxidized areas adjacent to the growing roots of the rice plant. This affect was found to occur in the lower reduced layer of rice fields.

The oxidizing "power" of the rice root can be very important in oxidizing toxic  $H_2S$  to a nontoxic form or to render high concentrations of soluble reduced iron and manganese to insoluble ferric and manganic oxides, thus possibly reducing toxic concentrations of the metallic ions. On the other hand, a stable metallic sulfide such as  $HgS$ , may be rendered more available and reactive due to the oxidative capacity of the root,

thus recycling a very toxic (mercury) element back into the ecological environment. Zinc and copper, elements necessary to the nutrition of the rice plant, readily form insoluble and unavailable sulfides in reduced environments. To achieve proper nutrition, the rice plant must be able to create a condition where the metal sulfides are transformed to more soluble and available forms for uptake by the plant.



## MATERIALS AND METHODS

### A. Soil Preparation

The soil used for these studies was a Crowley silt loam obtained from the Louisiana State University Rice Experiment Station at Crowley, Louisiana. All soil samples were air-dried, ground, passed through a 2 mm sieve, and thoroughly homogenized prior to their use in the experiments. Organic matter, as an energy source to promote microbial activity, was added to the soil. Ground rice straw (<35 mm) was used as the organic matter. The organic matter, at rates of 0.5% or 0.2% by weight of soil, was thoroughly mixed with the soil on a roller mixer.

Sodium sulfate was used in all experiments requiring the addition of sulfur. Rates and mode of sulfate application will be described individually for each experiment. In the experiments requiring radioactive sulfur, carrier free  $^{35}\text{S}$  as  $\text{H}_2^{35}\text{SO}_4$  and  $\text{Na}_2^{35}\text{S}$  were used. The experiments that required radioactive mercury received  $^{203}\text{Hg}$  as carrier free  $^{203}\text{HgCl}_2$ . The mode of application of the radioactive nuclides will be discussed separately for each experiment.

## B. Obtaining Anaerobic Conditions

In the experiments where complete anerobiosis was required, air-dried soil was weighed into 160 cc serum bottles with an equal amount of distilled water. The bottles were sealed with rubber serum caps. The serum cap was punctured with a hypodermic needle and a vacuum was applied to the serum bottles with a vacuum pump. A supply of oxygen-free argon was connected to the bottle by means of a 3-way valve. After a vacuum had been created within the serum bottles, the pump was closed off and argon was allowed to flow into the bottles until atmospheric pressure was obtained. This procedure was repeated several times, the result being the removal of entrapped air bubbles from the soil and the replacement of air in the bottles with argon. A similar procedure was followed when samples were contained in a vacuum desiccator. The samples were then incubated at 30°C.

## C. Sulfide Determination

The method used for the determination of total sulfide-sulfur was a modification of an iodometric method presented in Standard Methods for the Examination of Water and Wastewater (Farber, 1960). Total sulfide includes  $\text{HS}^-$ , as well as acid-soluble metallic sulfides present in the suspended matter. The ion  $\text{S}^{=}$

is not present in significant amounts below a pH of 13.0. Acid-insoluble sulfides are not detected by the tests used. Copper sulfide is a common acid insoluble sulfide.

The first step in the procedure for total sulfide analysis involved the transfer of a submerged, highly reduced soil from a serum bottle to a 250 ml gas washing bottle which had previously been purged with argon to displace oxygen. About 10 ml of distilled water was used to wash the serum bottles during the transfer process. This transfer was done as quickly as possible to keep atmospheric oxidation to a minimum. The gas washing bottle was fitted with a 2-hole top carrying a gas inlet tube and an outlet tube. The outlet was connected to a fritted-glass diffusion tube in another gas washing bottle. The washing bottle contained 10 ml of 2 N zinc acetate solution in 190 ml of distilled water. The apparatus was linked together with suitable connections so that gas could be passed through the system. Argon was bubbled through the soil suspension for 5 minutes to displace any oxygen prior to acidification of the soil suspension.

For determining total sulfide, the samples were acidified by introduction of 15 ml of concentrated  $\text{H}_2\text{SO}_4$ . The acidification step was done quickly to minimize air oxidation or loss of  $\text{H}_2\text{S}$ . Argon under a

pressure of about 7 psi was bubbled through the system for one hour. Complete mixing of the soil and acid was achieved with a magnetic stirrer which was in continuous operation. Upon acidification, soil sulfides were liberated as  $H_2S$ . The  $H_2S$  was swept into the gas washing bottle containing zinc acetate where it was precipitated as zinc sulfide.

An aliquot of 0.025 N iodine containing iodine in excess of that required to react with the collected sulfide was added to the zinc sulfide solution; 1.00 g of 0.025 N iodine is equivalent to 0.400 mg of sulfide. The solution was acidified with 15 ml concentrated HCl, shaken, and the excess iodine titrated by weight with 0.025 N  $Na_2S_2O_3$  using starch as the indicator. A blank determination was made at regular intervals.

From the volume of  $Na_2S_2O_3$  required to reach the starch endpoint, the concentration of sulfide could be calculated from the formula parts per million sulfide as  $S = \frac{(\text{ml iodine} - \text{g sodium thiosulfate}) \times 400}{\text{Weight of sample}}$

#### D. Measurement of Oxidation-Reduction Potential

In portions of this study, oxidation-reduction potential (redox potential,  $E_h$ ) measurements were made on the submerged soils. The measurements were made on parallel samples for each treatment by means of a bright

platinum electrode sealed in the side near the bottom of 30 x 250 mm Pyrex test tubes. A saturated calomel half-cell was used for a reference cell and potential measurements were made at regular intervals on a Beckman Zeromatic pH meter. Prior to commencing the experiment, the platinum electrodes were cleaned electrolytically in normal HCl. This was done by connecting the positive pole of a 12-volt wet-cell battery to a carbon electrode and connecting the negative pole of the battery to the platinum electrode and allowing hydrogen to bubble from the platinum electrode for three minutes (Redman and Patrick, 1965). The electrodes were then checked for accuracy in buffered quinhydrone solutions. The reference cell was connected into the test tube by means of a salt bridge containing saturated KCl and 2% agar.

E. Sulfide Evolution from Rice Straw Radiolabelled  
With  $^{35}\text{S}$

An experiment was performed to study the rate of production of sulfide from organic sulfur contained in plant material (ground rice straw) as compared to the rate of production of sulfide from an inorganic chemical source ( $\text{Na}_2\text{SO}_4$ ). The isotope  $^{35}\text{S}$  was used to trace the source of sulfide produced.

To incorporate the  $^{35}\text{S}$  into the plant material, rice was grown in sulfur-free sand culture until sulfur deficiency symptoms were evident. Then, carrier-free  $^{35}\text{S}$ -sulfate was added to the solution culture and incorporation was evidenced by the disappearance of the sulfur deficiency symptoms by the rice plants. The aerial plant material was then harvested, dried at  $70^{\circ}\text{C}$  and ground to pass a 35 mesh sieve.

In this study, two series of 50 g soil samples were incubated with an equal amount of distilled water in 160 cc serum bottles under anaerobic conditions at  $30^{\circ}\text{C}$ . One series received 0.5%  $^{35}\text{S}$  labelled plant material. Inorganic nonlabelled sodium sulfate was added to each sample of this series with the floodwater at a rate of 100 ppm sulfur. Therefore, labelled sulfide in this experiment could only evolve from the organic matter.

The other experimental series contained nonlabelled ground rice straw to provide the same amount of plant material. In this series, the added inorganic sodium sulfate was tagged with radioactive sulfate  $^{35}\text{S}$  ( $\text{Na}_2^{35}\text{SO}_4$ ) and was added with the floodwater. The inorganic sulfur source was added at a rate of 100 ppm. Thus, labelled sulfide in this series could only evolve from the inorganic source.

The soil was analyzed for total sulfide at regular intervals during a 50-day incubation period. The radioactive sulfide produced was assayed on a 2 ml aliquot of the zinc acetate-zinc sulfide suspension. The aliquot was taken prior to the iodometric titration step of the sulfide determination. The 2 ml aliquot containing  $^{35}\text{S}$  from the organic or inorganic sulfur source was air-dried in a 50 mm diameter aluminum planchet and radioassayed with a flowing gas proportional G-M counter. The counting time was 10 minutes. All radioassay procedures were carried out in an identical manner to obtain counts that could be compared on a relative basis. The radioassay was carried out at the completion of the study to compensate for half-life disintegration.

Oxidation-reduction potential measurements were made on identical parallel samples of each series. The pH changes were also monitored using a Corning combination microelectrode with a Beckman Zeromatic pH meter.

A subsample of radioactively labelled plant material was wet digested over heat using 5:2 nitric:perchloric acid, brought up to volume and an aliquot of the digest counted to determine the total radioactivity of the plant material. Another subsample of the labelled plant material was extracted with distilled water and

a 2 ml aliquot of the extract was radioassayed for water-soluble  $^{35}\text{S}$  to determine the inorganic sulfur in the plant material.

F. Sulfide Produced from Various Sulfur Sources

A study was initiated to evaluate microbial sulfide production from various inorganic sources of sulfur in a submerged soil. The sulfur sources for this study were: manganous sulfate, ferrous sulfate, cupric sulfate, silver sulfate, barium sulfate, calcium sulfate, lead sulfate, magnesium sulfate, sodium sulfate, and elemental sulfur. A soil without additional sulfur was included for comparison. Twenty subsamples, 50 g each, of each soil treatment were incubated anerobically with an equal weight of distilled water in 160 cc serum bottles at  $30^{\circ}\text{C}$  for 130 days. All of the sulfur sources were added at the rate of 100 ppm S and 0.5% organic matter was added as an energy source. Each sulfur source and organic matter addition was thoroughly mixed with a separate 2000 g bulk soil sample by means of a roller mixer. Total sulfide content of each treatment was determined on the 50 g subsamples at regular intervals for the duration of the experiment.



G. Sulfide Production as Affected by Various Oxidants  
Added to a Soil Prior to Submerging

This experiment was designed to investigate the effect of adding various oxidizing materials on sulfide production in a flooded Crowley silt loam. The oxidants were mixed with the air-dry soil prior to the addition of the floodwater. The following oxidants were used: oxygen, hydrated ferric oxide, potassium nitrate, manganese dioxide, ferricitrophosphate (a soluble organic ferric iron compound), and ferric phosphate. The oxidants were thoroughly mixed by use of a roller mixer with a 1000 g bulk sample of the soil at amounts equivalent to 300 and a 1000 ppm  $O_2$ . The  $O_2$  equivalency of the added oxidant was calculated by determining the ratio of the maximum number of electrons accepted by oxygen in a redox reaction, compared to that accepted by the oxidant. As an example,  $O_2 + 4e^- \longrightarrow 2 O^{=}$ , and  $NO_3^- + 5e^- \longrightarrow 1/2 N_2$  gives a ratio of 4/5. The ratio was then incorporated into a formula where: ppm  $KNO_3$  equivalent to  $O_2 = 4/5 \cdot$

$\frac{\text{mol. wt. } KNO_3}{\text{mol. wt. } O_2} \cdot \text{ppm } O_2 \text{ equivalency desired.}$

The soils containing the 300 ppm oxygen equivalent treatments received 100 ppm sulfur as  $Na_2SO_4$  and the soils containing the 1000 ppm oxygen equivalent oxidant treatments received 200 ppm sulfur as  $Na_2SO_4$ . The

oxygen was added at 300 ppm  $O_2$  utilizing a hypodermic syringe to add the required volume of air to give the rate of  $O_2$  desired. Separate 50 g soil samples of each treatment were weighed into separate 160 cc serum bottles with an equal amount of distilled water containing the sulfate sulfur. All of the soil samples were incubated anaerobically at  $30^{\circ}C$  with 0.5% organic matter added as an energy source. As a control series, a soil containing all the amendments except the oxidant was set up. The total sulfide concentration of each treatment series was determined at regular intervals during a 23-day incubation period. Oxidation-reduction potential measurements were made on all treatments for the duration of the study. A rate equation for sulfide evolution was calculated for each treatment. The equation used was  $Y = MX + b$ ; where Y and X are the coordinates of a point on the sulfide evolution curve, b is the intercept of the sulfide curve on the Y axis, and M is the rate of sulfide evolution. The equation was fitted to the data by the linear regression of ungrouped data method. Only the linear portion of the sulfide curve was used.

H. Sulfide Production as Affected by Various Oxidants  
Added to a Reduced Submerged Soil after Maximum  
Sulfide Formation

This investigation was initiated to study the effects of various oxidants on sulfide production in an anaerobic soil in which the oxidizing compounds were added after sulfide formation was a maximum. Two hundred ppm sulfate sulfur ( $\text{Na}_2\text{SO}_4$ ) was added to each soil sample to create a high sulfide content. The oxidants added were: oxygen, potassium nitrate, manganese dioxide, ferricitrophosphate and ferric phosphate. The oxidants were added in amounts equivalent to 500 and 1000 ppm  $\text{O}_2$ . Oxygen was added by use of a hypodermic syringe and needle at 500 ppm  $\text{O}_2$  only. Separate 50 g samples of Crowley silt loam containing 0.5% added organic matter and 50 ml distilled water containing the sulfate sulfur were incubated anaerobically in 160 cc serum bottles at  $30^\circ\text{C}$ . The oxidant treatments were separately placed in 10 x 75 mm test tubes inserted inside the serum bottles. The sealed oxygen-free bottles were then incubated for sufficient time (21 days) to allow maximum reduction and sulfide accumulation. Oxidation-reduction potential measurements were made on duplicate samples. The oxidants were mixed with the highly reduced soil by vigorously shaking the bottles on a reciprocal

shaker for 15 minutes. Total sulfide content of the treated soils was determined at regular time intervals during a 72-hour incubation period. An untreated (no oxidant) series was processed through the entire analytical procedure to evaluate the possibility of atmospheric oxygen contamination and subsequent oxidation of the sulfide.

#### I. Heavy Metal Sulfide Reactions in Aerobic and Anaerobic Soils

This investigation was conducted to evaluate the effects of aerobic and anaerobic soil conditions on the oxidation and subsequent solubilization and recycling of heavy metal sulfides. The metal sulfides under study were manganous sulfide ( $\text{MnS}$ ), ferrous sulfide ( $\text{FeS}$ ), zinc sulfide ( $\text{ZnS}$ ), cuprous sulfide ( $\text{CuS}$ ) and mercuric sulfide ( $\text{HgS}$ ). These very low water-soluble compounds are listed in decreasing order to their solubility. Each sulfide salt was radiolabelled with  $^{35}\text{S}$ . The oxidation of the sulfide salts was studied by an extraction of the labelled soils with water to remove soluble  $^{35}\text{S}$  resulting from the oxidation and subsequent dissolution of the salts. A subsequent radioassay of the water extract for  $^{35}\text{S}$  was carried out. Krauskopf (1967) and Garrels and Christ (1965)

gave several mechanisms for the chemical oxidation of metal sulfides in which the sulfur as sulfide was oxidized to the sulfate form. Based partially on this information it was considered that water-extractable  $^{35}\text{S}$  would give a good indication of the oxidation of the water-insoluble metal sulfide salts.

The metal sulfide salts were synthesized by adding dropwise into a 0.2 M solution of the chloride salt of the heavy metal a 0.1 M  $\text{Na}_2\text{S}$  solution tagged with  $^{35}\text{S}$ . All of the metal sulfide salts were synthesized in this manner. The precipitation was carried out in an excess of the metal chloride solution to insure that most of the  $^{35}\text{S}$  was precipitated as the metal sulfide salt. After precipitation, the salts were crystalized on a hot water bath at  $90^\circ\text{C}$  and washed repeatedly with distilled water until a negative chloride test was obtained. The salts received 35 to 40 washings. Between washings, the salts were returned to the hot water bath for settling prior to decanting the supernatant. The metal sulfide salts were then dried in a vacuum desiccator under an argon atmosphere. After drying, the salts were thoroughly ground and mixed with equal amounts of soil (Crowley silt loam). The grinding and mixing was conducted in a plastic glovebag in an argon atmosphere to prevent air oxidation and to prevent leakage of the labelled sulfide. The five

radioactive sulfide salts were mixed with large quantities of air-dried Crowley silt loam on a roller mixer to give total weights of 1000 g of soil per treatment. The air-dried soils were stored under argon. Ground rice straw at a rate of 0.2% by weight of soil was added during the mixing operation to each of the five treated soils to serve as a microbial energy source. After completion of the soil treatment, portions of each treated soil were weighed into 40 separate 15 x 125 mm test tubes. Each tube held 10 g of air-dried soil. Each treated soil was then incubated at 30°C under two conditions. One condition was aerobic where the soil was kept at optimum field moisture (1/3 bar moisture tension), thus allowing the large soil pores to be free of water and be open to oxygen movement. Two and one-half ml of water per 10 g of soil were found to be sufficient for this treatment. The other samples were incubated under anaerobic conditions obtained by flooding the 10 g soil samples with 10 ml distilled water. The soils were incubated for 75 days with tubes being sampled at regular intervals for analysis.

The oxidation of the sulfide salts to higher oxides of sulfur was traced by a water extract of each soil at a soil water ratio of 1:1 and subsequent centrifugation of each soil solution until a clear supernatant was

obtained. The extraction procedure was carried out in the original tubes containing the treated soil. The aerobic soils received 7.5 ml water and the flooded soils received no additional water. A 2 ml aliquot was obtained from each sample and was stored until time for radioassay for water-soluble  $^{35}\text{S}$  arising from the metal sulfides. The water extract would remove only the oxidized (water-soluble) sulfur forms and leave the insoluble sulfide salts.

The radioassay for the  $^{35}\text{S}$  was accomplished using liquid scintillation counting procedures. The 2 ml aliquot from each treatment was mixed with 15 ml of "Aguasol" (obtained from New England Nuclear) and was assayed for 10 minutes with a Beckman L. S. 250 Liquid Scintillation Analyzer. A variable discriminator was used with the lower window at 20 and the upper window at 420. All samples including standards were assayed in an identical manner to achieve proper relative counting of the  $^{35}\text{S}$ . All of the samples and dosing standards were radioassayed at the completion of the study, consequently, a half-life disintegration correction was not needed. The samples as well as the dosing standards were derived from the same source of  $^{35}\text{S}$ , therefore, were at the same point of disintegration.

The total radioactivity of each treated soil (containing synthesized sulfide salts) was determined by wet digestion over heat of the respective soil treatments with nitric:perchloric acid (5:2) and subsequent radioassay at the completion of the study. After complete digestion, where the acid digest was brought to near dryness, the samples were made up to a volume with distilled water and subsamples were radioassayed in a fashion identical to the assay of the water extracts of the treated soils. The oxidation of the metal sulfide was calculated and shown as a percent recovery of water-soluble  $^{35}\text{S}$  from the  $^{35}\text{S}$  labelled sulfide salt.

#### J. Reactions of Mercury in Aerobic and Anaerobic Soils

The previous experiment had placed special emphasis on the oxidation and movement of sulfides from heavy metal sulfide salts. This study placed special emphasis on the solubilization and movement of mercury from mercuric sulfide incubated in aerobic and anaerobic soil. The isotope  $^{203}\text{Hg}$  was incorporated into the  $\text{HgS}$  salt and a water extract of the soils was used to measure the changes in the soluble  $^{203}\text{Hg}$  arising from the insoluble sulfide salt. An increase of water-extractable  $^{203}\text{Hg}$  would be indicative of an oxidation to a more soluble form. Wiklander (1969) postulated that



mercury sulfide was oxidized under aerobic conditions to the more soluble mercury sulfate.

Mercury sulfide labelled with  $^{203}\text{Hg}$  was synthesized by adding 0.1 M  $\text{HgCl}_2$  containing  $^{203}\text{Hg}$  dropwise into 0.2 M  $\text{Na}_2\text{S}$  forming a mercury sulfide precipitate. An excess of  $\text{Na}_2\text{S}$  was used to insure that most of the radioactive mercury was precipitated. The  $^{203}\text{Hg}$  labelled  $\text{HgS}$  salt was processed exactly as the sulfide salts in the previous experiment. Separate 10 g samples of the treated soils were placed in 15 x 125 mm test tubes and incubated for 70 days at  $30^\circ\text{C}$  under two conditions. The soil also contained 0.2% ground rice straw. The first condition was anaerobic where each soil was continuously flooded with 10 ml distilled water. The second condition was where the soil was kept moist (1/3 bar moisture tension) for 40 days and then flooded with enough distilled water to give a total of 10 ml added water. The soil was then kept flooded for the remainder of the study.

The soils were extracted for water-soluble  $^{203}\text{Hg}$  by the procedure described in the previous experiment. A 4 ml aliquot containing  $^{203}\text{Hg}$  a gamma emitter, was radioassayed for relative counts with a deepwell  $\text{NaI(Tl)}$  crystal scintillation detector. Each sample was counted for 30 minutes. All of the samples and standards were assayed after the completion of the study. The increase

of soluble mercury from the mercury sulfide was determined and presented as counts per minute  $^{203}\text{Hg}$  per gram of labelled soil. The percent recovery of  $^{203}\text{Hg}$  from the labelled soil was also calculated.

Previously, oxidation and subsequent solubilization of insoluble mercury sulfide placed in moist and flooded soils had been studied. In many documented cases (Wallace et al., 1971), mercury was placed into flooded environments as the soluble and very toxic  $\text{HgCl}_2$ . Mercury has been postulated to enter into the dynamic oxidation-reduction systems presented in flooded sediments and soils and in many cases be rendered less soluble as the sulfide salt (Geological Survey Staff, 1970). This experiment was set up to study the changes in extractable  $^{203}\text{Hg}$  originating from  $^{203}\text{HgCl}_2$  placed into a flooded and aerobic soil environment. Normal ammonium acetate (pH 4.5) was used to extract the  $^{203}\text{Hg}$ , because a water extract would not remove the  $^{203}\text{Hg}$  adsorbed to the cation exchange complex. A decrease in extractable  $^{203}\text{Hg}$  under anaerobic conditions would be indicative of the transformation of the mercury into a more stable, insoluble form as well as being adsorbed into the soil exchange complex.

Carrier-free  $^{203}\text{Hg}$  as  $^{203}\text{HgCl}_2$  was thoroughly mixed with a Crowley silt loam containing 0.2% added organic matter. Separate 10 g subsamples were weighed into

15 x 125 mm test tubes and flooded with 10 ml distilled water. The treated soil was incubated at 30°C for 70 days. Samples were taken on a regular basis for evaluation. To evaluate the recovery of the  $^{203}\text{Hg}$ , the soil samples were extracted with 30 ml of normal ammonium acetate (pH 4.5). The extraction was done by mixing triplicate 10 ml portions of the ammonium acetate with the soil, centrifugation of each replicate and collection of the extract. The extraction procedure was conducted over a 6-hour period of time.

The relative radioassay of the  $^{203}\text{Hg}$  was done as described in the previous study. The recovery of  $^{203}\text{Hg}$  was presented as counts per minute  $^{203}\text{Hg}$  per gram of soil and percent recovery of the added  $^{203}\text{Hg}$ .

An additional experiment was conducted to evaluate the effect of a microbially active flooded soil and a sterile flooded soil on the removal of soluble  $^{203}\text{Hg}$  added to the overlying floodwater of the soils. The radioactive mercury was added to the floodwater overlying the soils and the concentration of mercury in the floodwater was followed for 25 days. Quadruplicate 1000 g samples of Crowley silt loam with 0.2% added organic matter were each placed in 2 liter Erlenmeyer flasks and flooded with 1000 ml distilled water. The soils were then incubated at 30°C for 3 weeks until reducing conditions were evident. Two of the soils

were then sterilized once in a steam autoclave for one hour at  $120^{\circ}\text{C}$  and 20 psi pressure. The soils were then reincubated at  $30^{\circ}\text{C}$  for 2 weeks to insure sterility. Separate 25 ml aliquots containing equal amounts of carrier free  $^{203}\text{HgCl}_2$  were then added to the floodwater of each of the four soils. Four ml aliquots from the floodwater were taken at regular intervals throughout the study. The aliquots were stored and then assayed for  $^{203}\text{Hg}$  as described previously. Sterile conditions were maintained throughout the experiment.

K. Effect of the Rice Plant on Metal Sulfides in a Submerged Soil

An experiment was initiated to study the oxidizing capability of the roots of rice plants with respect to several  $^{35}\text{S}$  labelled sulfide salts mixed in a flooded Crowley silt loam. The oxidation of the sulfide salts was measured by monitoring uptake of  $^{35}\text{S}$  by the rice plants at the duration of the study. The toxicity of sulfide to the rice plant is well documented (Sturgis, 1936; James, 1953; Okajima and Takagi, 1953; Ponnamperna, 1955; Mitsui and Kumazawa, 1964; Ford, 1965; Hollis, 1967) and it is highly unlikely that sulfide will be taken up by the rice plant but will be oxidized to  $\text{SO}_4^{=}$  or other oxides of sulfur in the oxygenated zone adjacent to the rice root. Work has shown (Okajima

and Takagi, 1955; 1956) that very little sulfide is taken up by the rice plant and the small amount of sulfide that moves into the root remained there. The metal sulfide salts used were: manganous sulfide ( $\text{MnS}$ ), ferrous sulfide ( $\text{FeS}$ ), zinc sulfide ( $\text{ZnS}$ ), cuprous sulfide ( $\text{CuS}$ ) and mercuric sulfide ( $\text{HgS}$ ). As a control, water-soluble sodium sulfide ( $\text{Na}_2\text{S}$ ) was used. The metal sulfide salts were labelled with  $^{35}\text{S}$  and prepared exactly as described in Section I. Organic matter as ground rice straw was added to the soil at a rate of 0.2% by weight of soil. The  $\text{Na}_2\text{S}$  labelled with  $^{35}\text{S}$  was acquired from International Chemical and Nuclear Corporation as carrier-free  $\text{Na}_2^{35}\text{S}$  and needed no further preparation. The  $\text{Na}_2^{35}\text{S}$  was applied directly to the air-dried soil as was the synthesized metal sulfides and the soil preparation was carried out under argon as previously described.

Ten 75 g replicates of each of the six labelled air-dried soils were weighed into separate 38 x 200 mm test tubes. The labelled soil was followed by a 50 g layer of unlabelled soil from the original bulk soil sample. This gave a depth of unlabelled soil of about 80 mm which would inhibit the movement of oxygen into the labelled soil layer where it could oxidize the labelled components. Patrick and DeLaune (1971) have previously shown that in a flooded soil exposed to the

atmosphere, oxidizing conditions with respect to sulfide oxidation progressed to a maximum depth of 12 mm after 18 weeks of incubation. An 80 mm buffer zone as described was used in this experiment. The soils were then submerged with water to a depth of about 2 to 3 cm and incubated at 30°C for 3 weeks. An incubation time of 3 weeks was considered sufficient to obtain reducing conditions that would insure the reduced state of the labelled sulfides. Rice (Oryza sativa, var. Saturn) was germinated in sand culture, grown for 3 weeks and transplanted into the test tubes containing the labelled soils. Eight plants were transplanted into each test tube and thinned to 6 plants after 3 days. The rice was grown under supplementary light for 21 days and the aerial portion of the plants was harvested. Due to poor initial growth after transplanting, the rice growing on the  $^{203}\text{HgS}$  labelled soil was grown for an additional 20 days to insure good root proliferation in the radioactive soil. The harvested plant material was then dried for 24 hours at 70°C in a forced air oven. Each replicate was weighed for yield measurements. The plant material was then ground and wet digested over heat using 5:2 nitric:perchloric acid. The digest was made up to volume and a 2 ml aliquot was radioassayed for  $^{35}\text{S}$  as described in Section I.

An air-dried subsample of each of the sulfide labelled soils was wet digested as described in Section I and an aliquot of the digest radioassayed for  $^{35}\text{S}$ . This gave the total relative radioactivity contained in each labelled soil. From the data, percent uptake of  $^{35}\text{S}$  by the rice plant from each metal sulfide was calculated. The percent uptake of  $^{35}\text{S}$  could be used as a measure of the oxidative capacity of the rice root. All radioassay procedures were conducted in an identical manner to insure relative comparison of the data. The samples and standards, as well as the soil digest were assayed at the termination of the study.

## RESULTS AND DISCUSSION

### A. Sulfide Evolution from Rice Straw Labelled With $^{35}\text{S}$

A study was conducted to evaluate sulfide production in soil from added organic material (ground rice straw) as compared to sulfide produced from an inorganic sulfur source ( $\text{Na}_2\text{SO}_4$ ). The isotope  $^{35}\text{S}$  was used to trace the source of the sulfide produced in a Crowley silt loam. Similar samples of soil were submerged with water with each series containing equal amounts of organic matter and inorganic sulfate-sulfur. However, one of the series of treated soil had inorganic  $^{35}\text{S}$  added to it and the other series contained organic material labelled with  $^{35}\text{S}$ . The results presented in Figure 1 depict a very rapid and complete evolution of sulfide from the inorganic sulfate-sulfur source. The rate curves of sulfide produced from the inorganic  $^{35}\text{S}$  ( $^{35}\text{SO}_4^-$ ) and the nonradioactive inorganic sulfur ( $\text{SO}_4^-$ ) exhibit a high degree of similarity. The inorganic  $^{35}\text{S}$  was added to the soil at a rate of 10,000 cpm  $^{35}\text{S}$  per 50 g soil. About 90% of both the added inorganic  $^{35}\text{S}$  and the non-labelled inorganic  $\text{SO}_4^-$  were recovered as sulfide. Sulfide production had reached a peak after 11 days of incubation. These data demonstrate the validity of using  $^{35}\text{S}$  to trace sulfide production in a flooded soil.



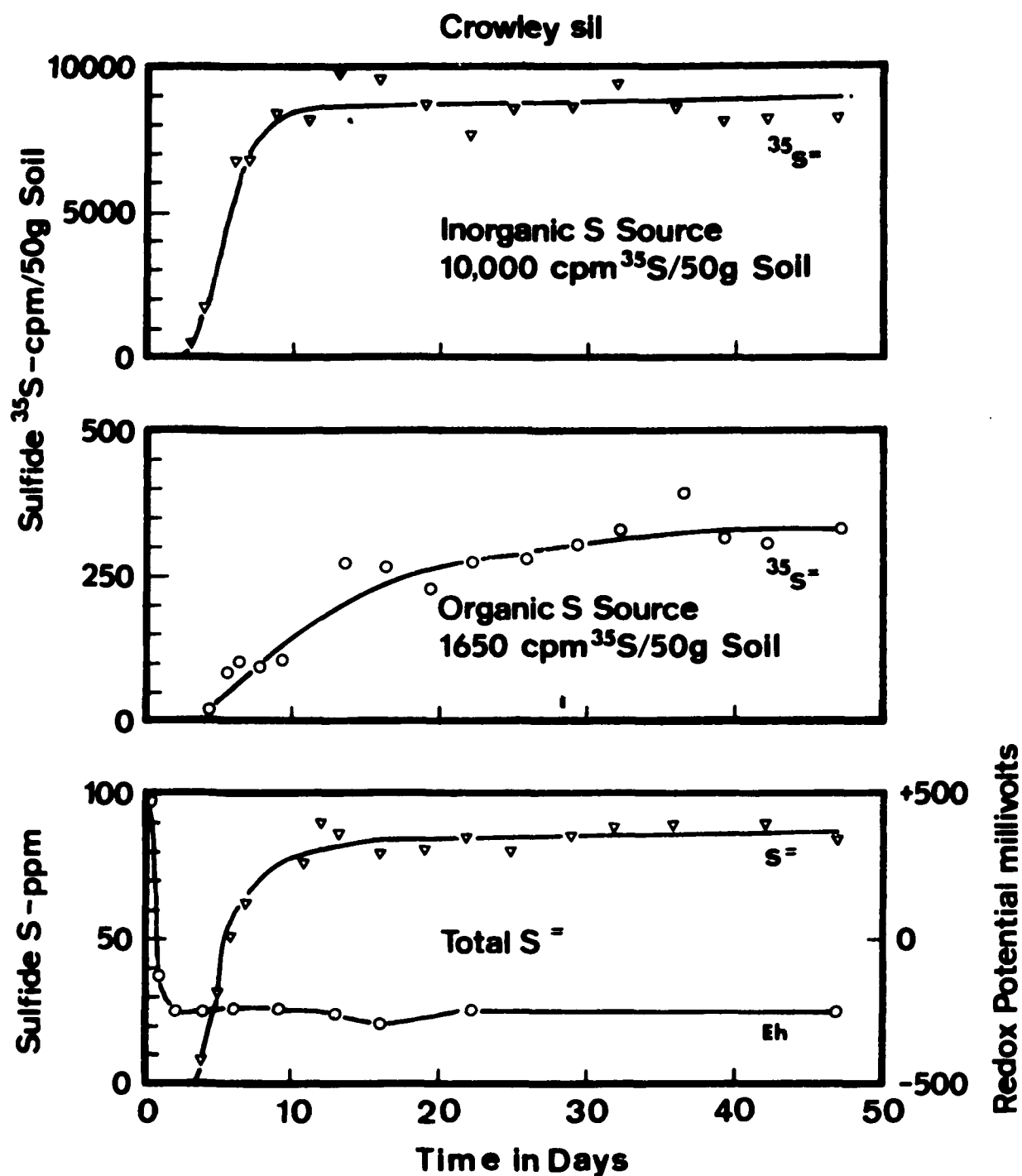


Figure 1. A comparison of sulfide production from an organic and an inorganic sulfur source in a flooded Crowley silt loam. Redox potential values are also shown.

The graph depicting sulfide evolution from the  $^{35}\text{S}$  labelled plant material presented in Figure 1 shows a slower and less complete evolution of sulfide in comparison to the inorganic sulfur source. The  $^{35}\text{S}$  labelled plant material contained 1650 cpm  $^{35}\text{S}$  per 50 g of soil and approximately 18% recovery of the  $^{35}\text{S}$  added as organic sulfur was achieved. The radioactively labelled plant material did not contain any appreciable amounts of water soluble  $^{35}\text{S}$  prior to adding it to the Crowley soil. The sulfide production had not reached a maximum rate at the end of the incubation period. The mineralization of organic sulfur to sulfide in an anaerobic environment may be an important source of sulfide in flooded soils, especially those high in organic matter.

#### B. Sulfide Produced from Various Sulfur Sources

An experiment was conducted to study the reduction of  $\text{SO}_4^{=}$  from various sulfate containing compounds placed in a flooded soil. Elemental sulfur was also used as a source of sulfur. The sulfur sources placed in the flooded soils were manganous sulfate ( $K_{sp} = 2.4 \times 10^3$ ), ferrous sulfate ( $K_{sp} = 3.3 \times 10^{-1}$ ), cupric sulfate ( $K_{sp} = 4.4 \times 10^2$ ), silver sulfate ( $K_{sp} = 1.2 \times 10^{-5}$ ), barium sulfate ( $K_{sp} = 1.1 \times 10^{-10}$ ), calcium sulfate ( $K_{sp} = 6.1 \times 10^{-5}$ ), lead sulfate ( $K_{sp} = 1.1 \times 10^{-8}$ ),

magnesium sulfate ( $K_{sp} = 1.8 \times 10^{-4}$ ), sodium sulfate ( $K_{sp} = 6.4 \times 10^{-1}$ ) and elemental sulfur. The results of this study are presented in Figure 2. The sources of sulfur varied in solubility (Garrels and Christ, 1965) and in their toxicity to the soil microbial population. Copper sulfate and silver sulfate appeared to create a toxic condition for the microorganisms as very little sulfide was evolved from the added or indigenous sulfur. Insoluble barium sulfate resulted in sulfide production only 4 to 5 ppm greater than that of the untreated soil. Elemental sulfur was fourth lowest in order of sulfide production with about 28% recovery of the added sulfur. Postgate (1951), in a culture study, found that only a small amount of elemental sulfur was reduced to sulfide by D. desulfuricans. The remaining sulfur sources resulted in high sulfide production. From 80 to 95 ppm sulfide was evolved which, when corrected for sulfide produced in the untreated soil represents 65 to 80% conversion of the added sulfate to sulfide. With the exception of toxic copper and silver sulfate and the very insoluble barium sulfate, sulfide production did not appear to be related to the solubility of the sulfate source.

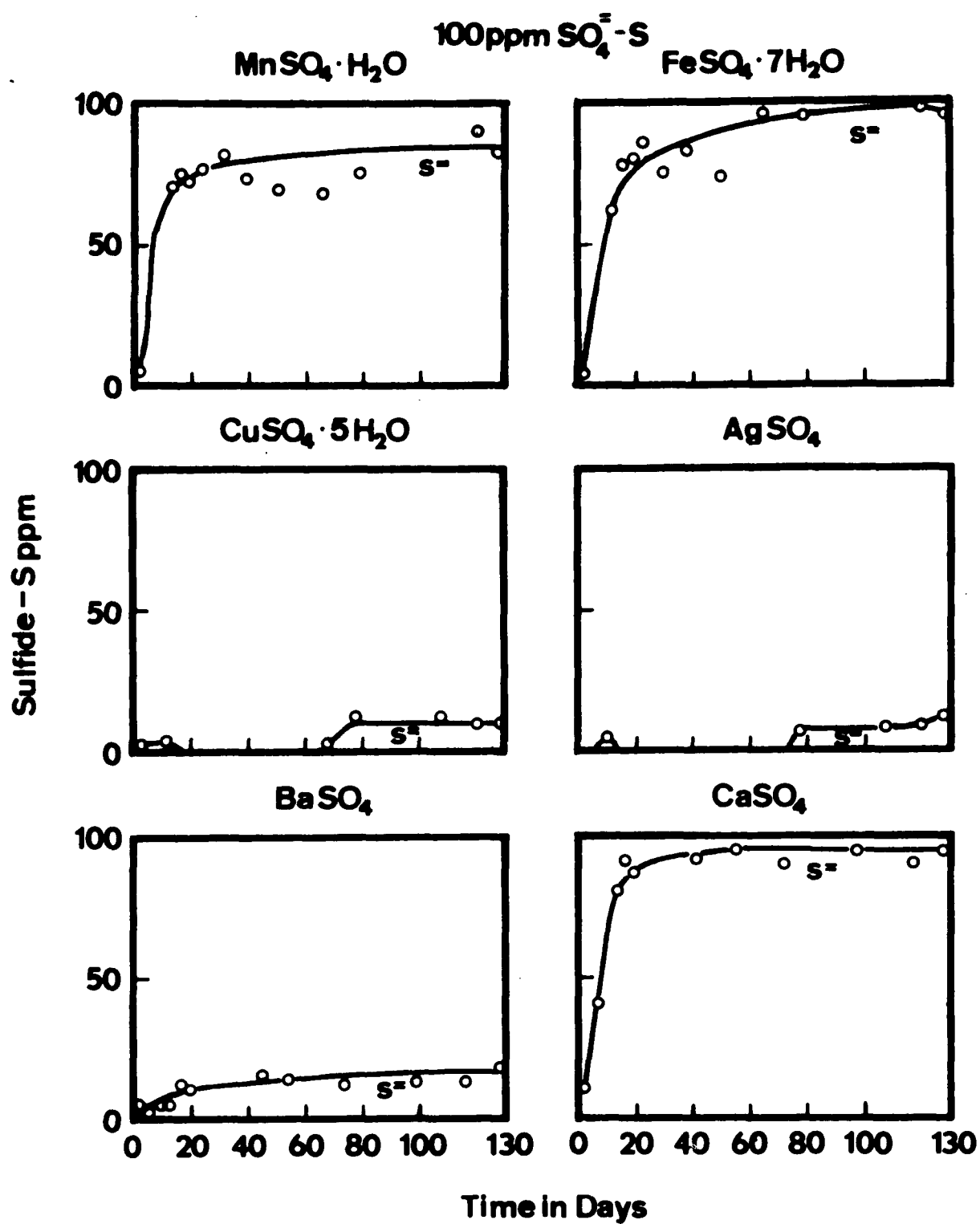


Figure 2. Sulfide production from various inorganic sulfur sources added to a flooded Crowley silt loam.

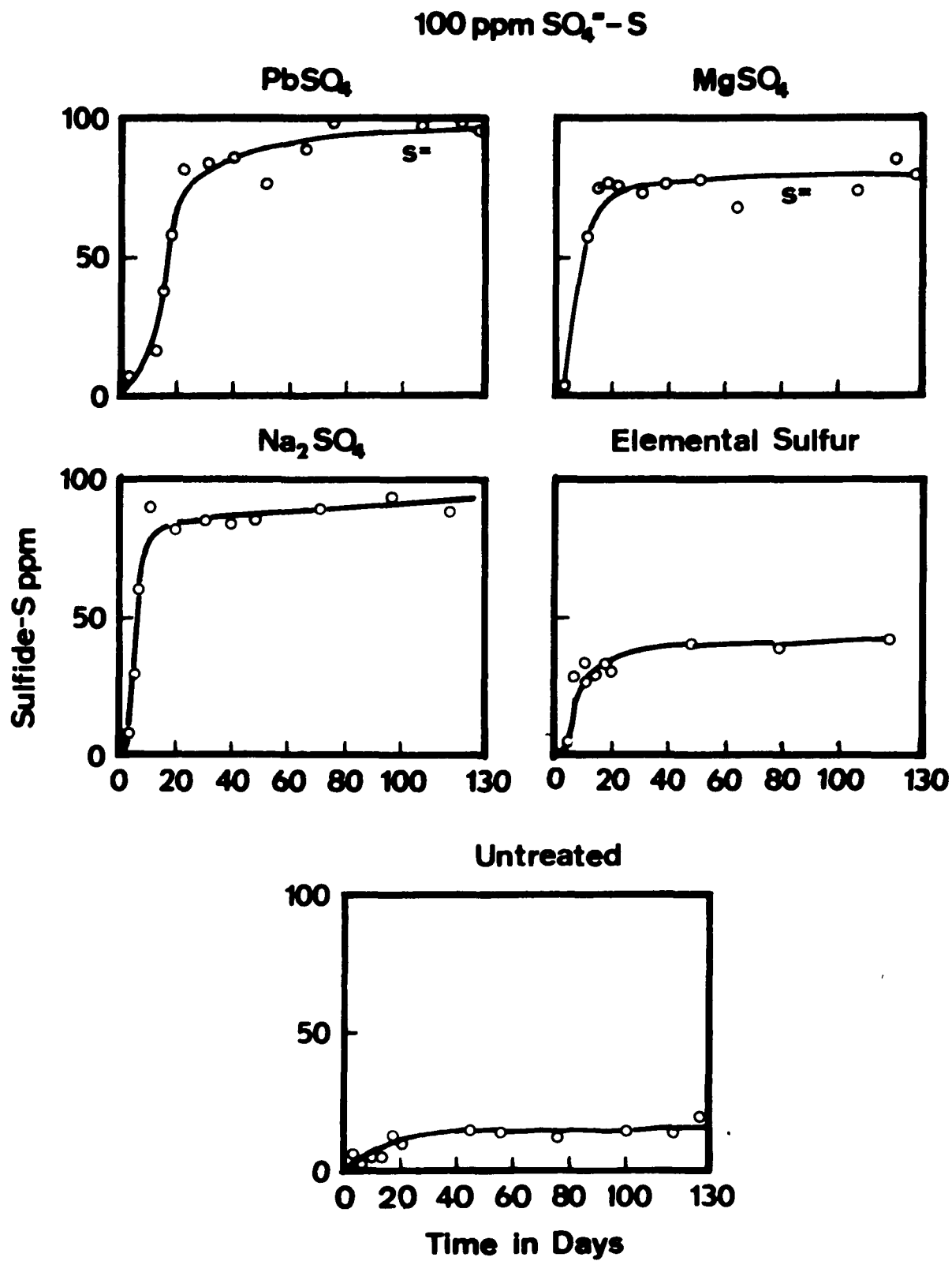


Figure 2. (Continued) Sulfide production from various inorganic sulfur sources added to a flooded Crowley silt loam.

C. Sulfide Production as Affected by Various Oxidants  
Added to a Soil Prior to Submerging

A study was conducted to evaluate the effect of adding various oxidizing compounds on sulfide production in a Crowley silt loam. The oxidants were added to the soil prior to flooding. The data illustrating an untreated control and the effects of additions of oxygen, ferric oxide, potassium nitrate, manganese dioxide, ferricitrophosphate, and ferric phosphate are presented in Figures 3 to 8.

The data in Figure 3 show the amount of sulfide produced following the addition of 100 and 200 ppm sulfate-sulfur as well as the respective redox potential measurements. The maximum rate of sulfide production was 16.6 and 29.6 ppm sulfide per day for the 100 and 200 ppm sulfur treatments. The rate of reduction of sulfate was apparently dependent on the concentration of sulfate in the flooded soil. This suggests that the mechanism responsible for the reduction of sulfate was not a limiting factor. Sulfide evolution was initiated on the third day of incubation and reached a peak after 10 days for both levels of sulfate. The redox potential fell to about -300 millivolts 3 days after incubation.

The oxygen treatment shown in Figure 4 reduced the maximum rate of sulfide production by 6.3 ppm per day.

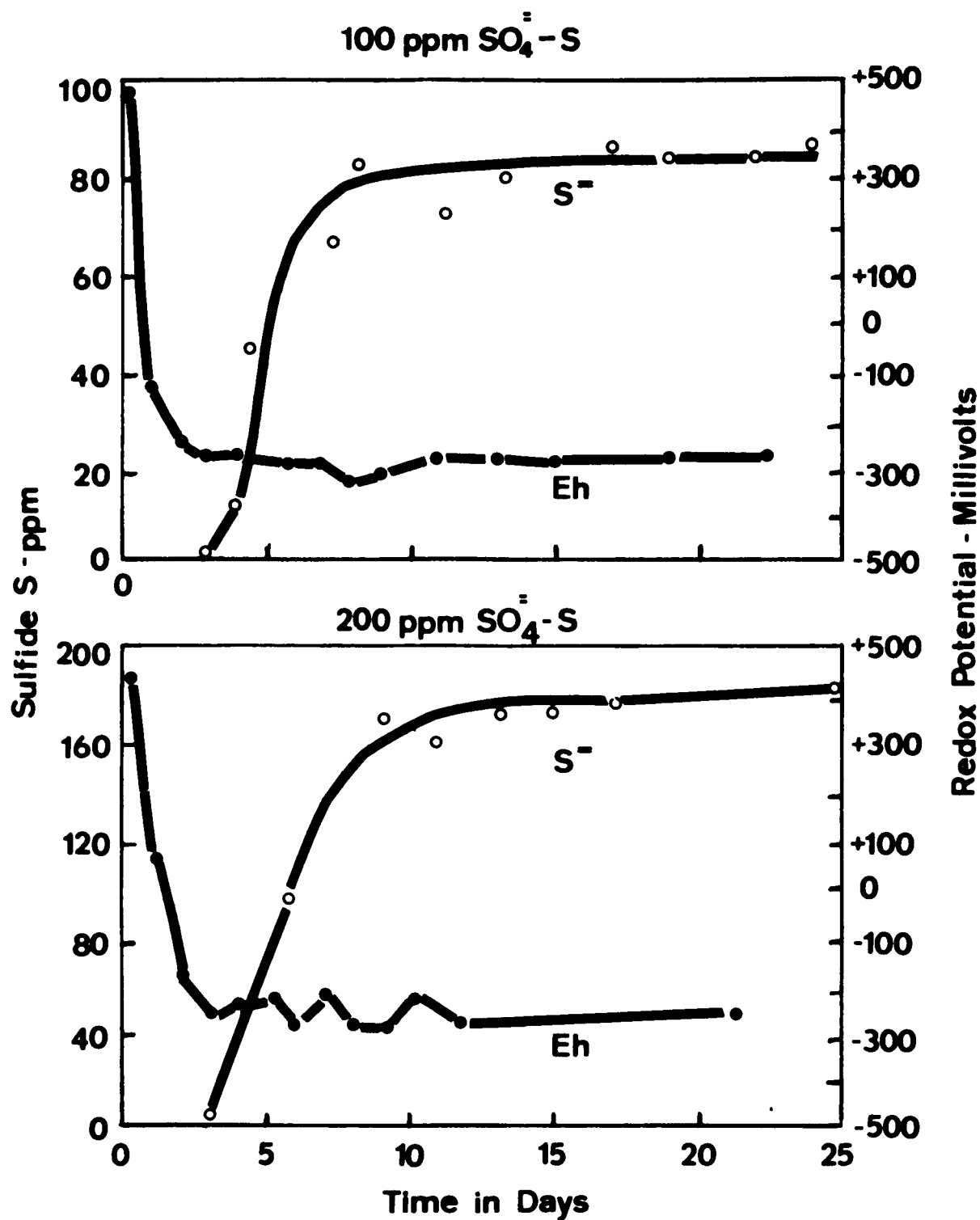


Figure 3. Sulfide produced from 100 and 200 ppm SO<sub>4</sub><sup>2-</sup>-S added prior to flooding a Crowley silt loam. Redox potential values are also shown.

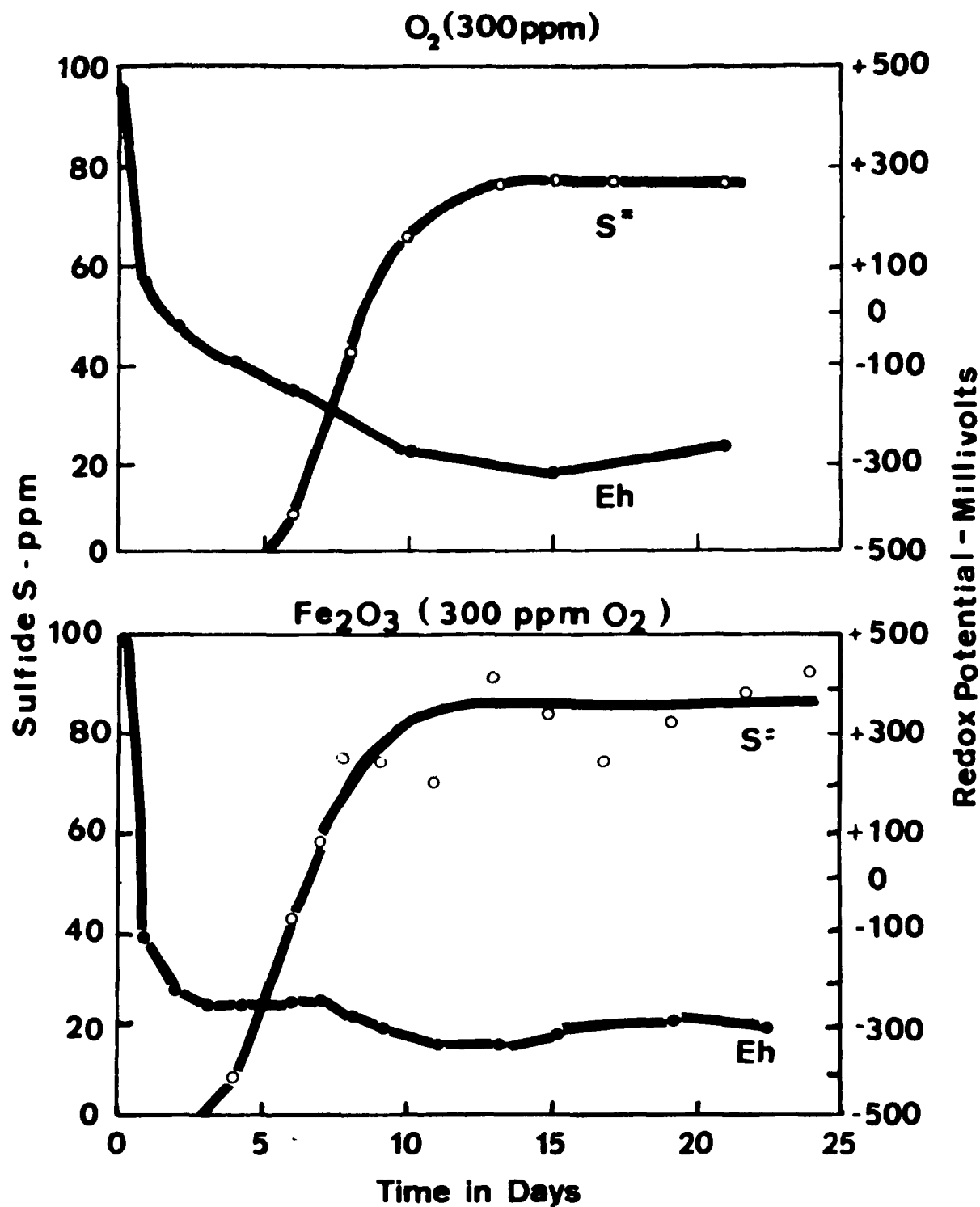


Figure 4. Sulfide produced from 100 ppm  $\text{SO}_4^{2-}\text{-S}$  as affected by the addition of 300 ppm  $\text{O}_2$  and ferric oxide, equivalent to 300 ppm  $\text{O}_2$ , prior to flooding a Crowley silt loam. Redox potential values are also shown.



The 300 ppm  $O_2$  also delayed the initial sulfide production by 2 days. Redox potential of the treated soil was held above normal (compared to the soil receiving no oxidant) for 10 days. The ferric oxide treatment equivalent to 300 ppm  $O_2$  shown also on Figure 4 decreased the maximum rate of sulfide evolution by 3.7 ppm sulfide per day. No delay in initial sulfide production was noted. Likewise, the redox potential was not affected, and decreased to -300 millivolts after 9 days.

Figure 5 represents the results of the potassium nitrate treatments at amounts equivalent to 300 and 1000 ppm  $O_2$ . The 300 ppm level reduced maximum sulfide production by 6.3 ppm per day and delayed the initiation of sulfide production by 1 day. Compared to the untreated soil, redox potential was above normal for 9 days. The 1000 ppm nitrate level did not appear to change the maximum rate of sulfide production as compared to the 300 ppm level. However, the initiation of sulfide production was delayed for 17 days. In contrast to the untreated soil and the 300 ppm treatment, the redox potential was held well above the minimum Eh level for 22 days. Upon the onset of sulfate reduction the redox potential had decreased to -250 millivolts. The 300 ppm level of nitrate appeared to be readily reduced and only slightly affected sulfide production and redox potential. The 1000 ppm level of nitrate,

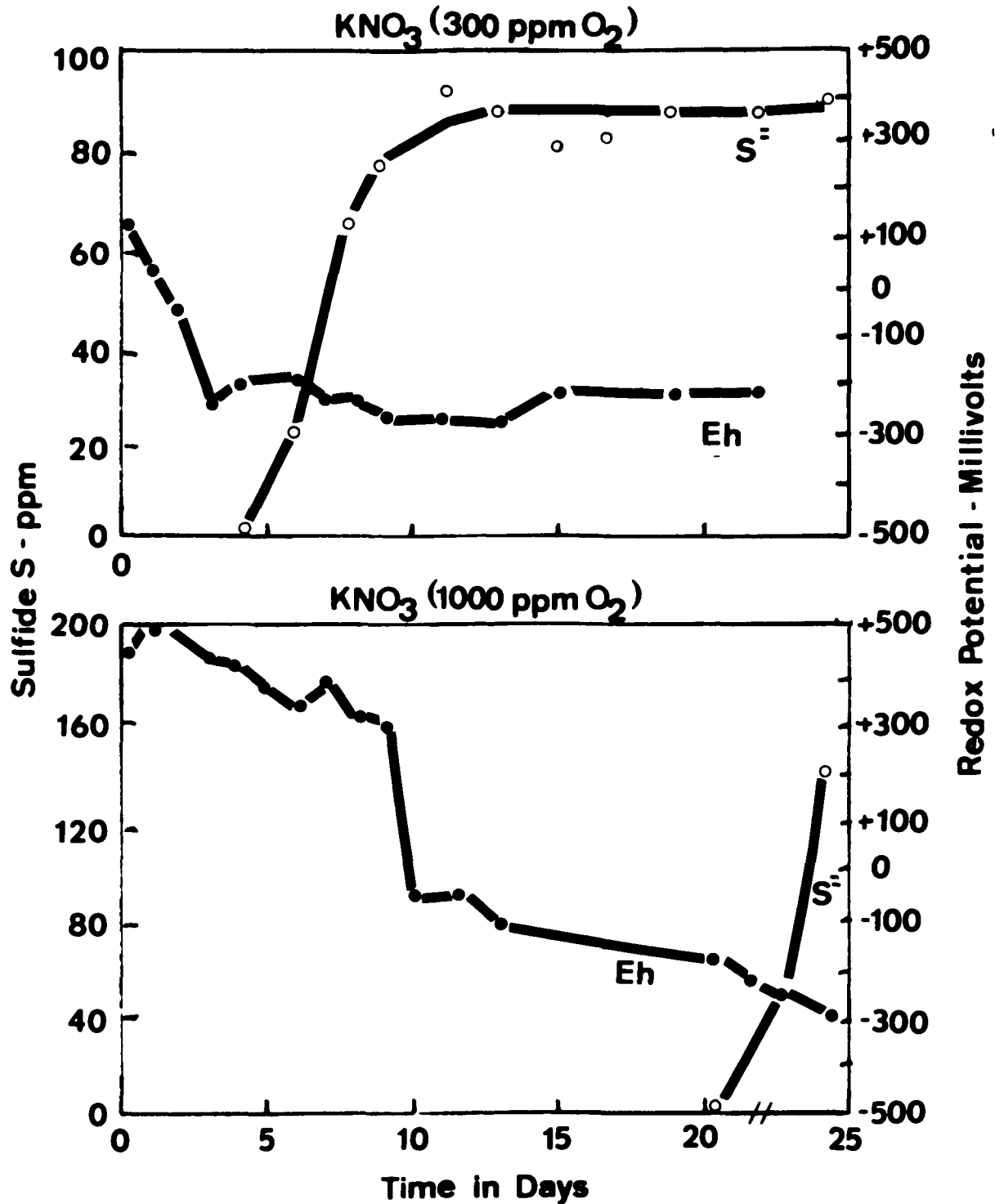


Figure 5. Sulfide produced from 100 and 200 ppm  $\text{SO}_4^{2-}\text{-S}$  as affected by the addition of potassium nitrate, equivalent to 300 and 1000 ppm  $\text{O}_2$ , prior to flooding a Crowley silt loam. Redox potential values are also shown.

on the other hand, contributed a large amount of readily reducible material which resulted in a much longer period of time when no sulfide was produced. Redox potential was similarly affected. However, in both treatments sulfide production was near maximum shortly after initial sulfide formation. This suggests that almost all of the nitrate had been reduced prior to sulfide production. Connell and Patrick (1969) found no sulfide formation until all nitrate had been reduced.

Results of the manganese dioxide treatments are shown in Figure 6. The treatment equivalent to 300 ppm  $O_2$  reduced the maximum rate of sulfide formation by 5.4 ppm per day. However, the initiation of sulfide production was not delayed. The redox potential was slightly elevated due to the presence of the oxidized material. The amount equivalent to 1000 ppm  $O_2$  reduced the maximum rate of sulfide evolution by 16.8 ppm per day. The high rate of manganese delayed the initial sulfide production by 3 days and the redox potential was maintained above that of the untreated soil for several days.

Figure 7 illustrates the treatment utilizing fer-ricitrophosphate to reduce and delay sulfide evolution. The amount equivalent to 300 ppm  $O_2$  resulted in a depression of 2.3 ppm sulfide per day in the maximum rate of sulfide produced. This treatment also delayed

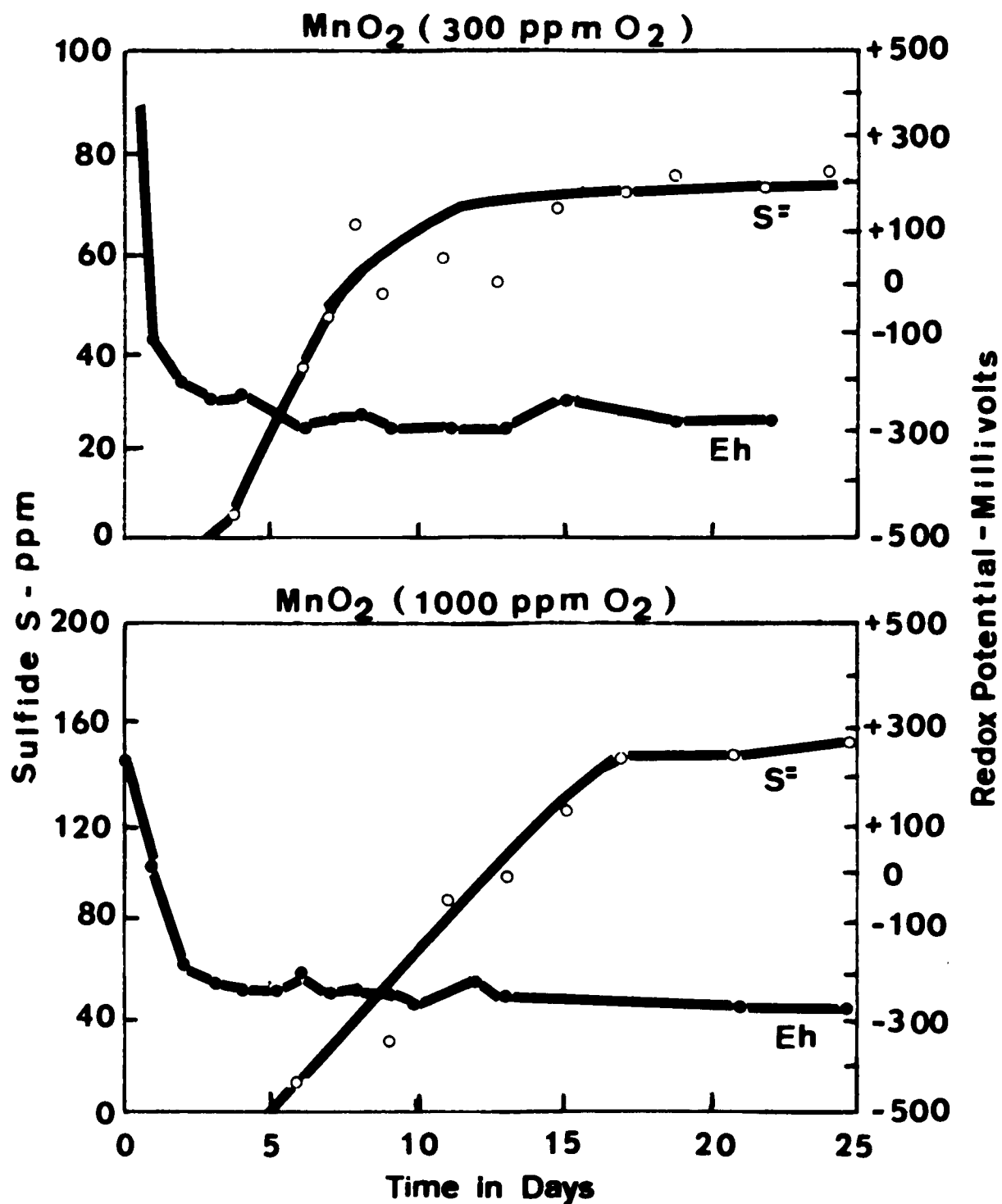


Figure 6. Sulfide produced from 100 and 200 ppm  $\text{SO}_4^{2-}\text{-S}$  as affected by the addition of manganese dioxide, equivalent to 300 and 1000 ppm  $\text{O}_2$ , prior to flooding a Crowley silt loam. Redox potential values are also shown.

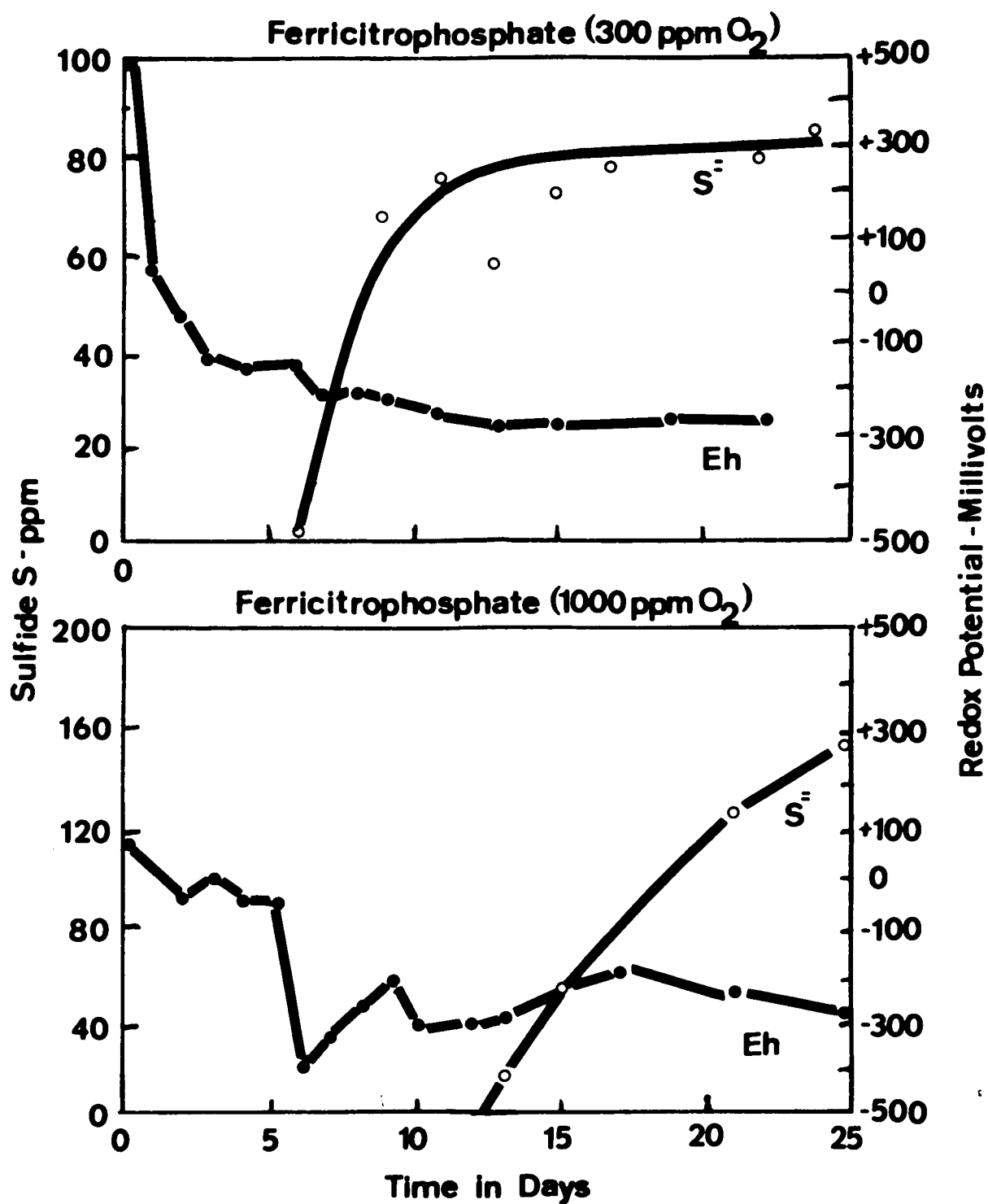


Figure 7. Sulfide produced from 100 and 200 ppm  $\text{SO}_4^{2-}\text{-S}$  as affected by the addition of ferricitrophosphate, equivalent to 300 and 1000 ppm  $\text{O}_2$ , prior to flooding a Crowley silt loam. Redox potential values are also shown.

initial sulfide evolution for 3 days. The redox potential was maintained above that of the untreated soil for about 8 days. The amount of oxidant equivalent to 1000 ppm  $O_2$  reduced the maximum rate of sulfide formation by 17.7 ppm per day. This treatment also delayed the initiation of sulfide evolution by 12 days. Redox potential measurements were very erratic and above normal for about 15 days. This soluble ferric iron compound affected sulfide formation and redox potential in a manner similar to the nitrate treatments, but the effect caused by the ferricitrophosphate was not as pronounced. This was probably due to the fact that nitrate can be reduced more readily and at a much higher redox potential than the ferric iron compound.

Figure 8 depicts the effect of ferric phosphate on sulfide formation and redox potential. The treatment equivalent to 300 ppm  $O_2$  reduced the maximum rate of sulfide production by 9.2 ppm per day. The initiation of sulfide formation was not affected. Redox potential was maintained slightly above that of the untreated soil for the first 5 days. The amount of ferric phosphate equivalent to 1000 ppm  $O_2$  reduced the maximum rate of sulfide formation by 24.1 ppm per day. Initiation of sulfide evolution was delayed by only 2 days. Redox potential was maintained above normal for about 10 days. Ferric phosphate appeared to be the most

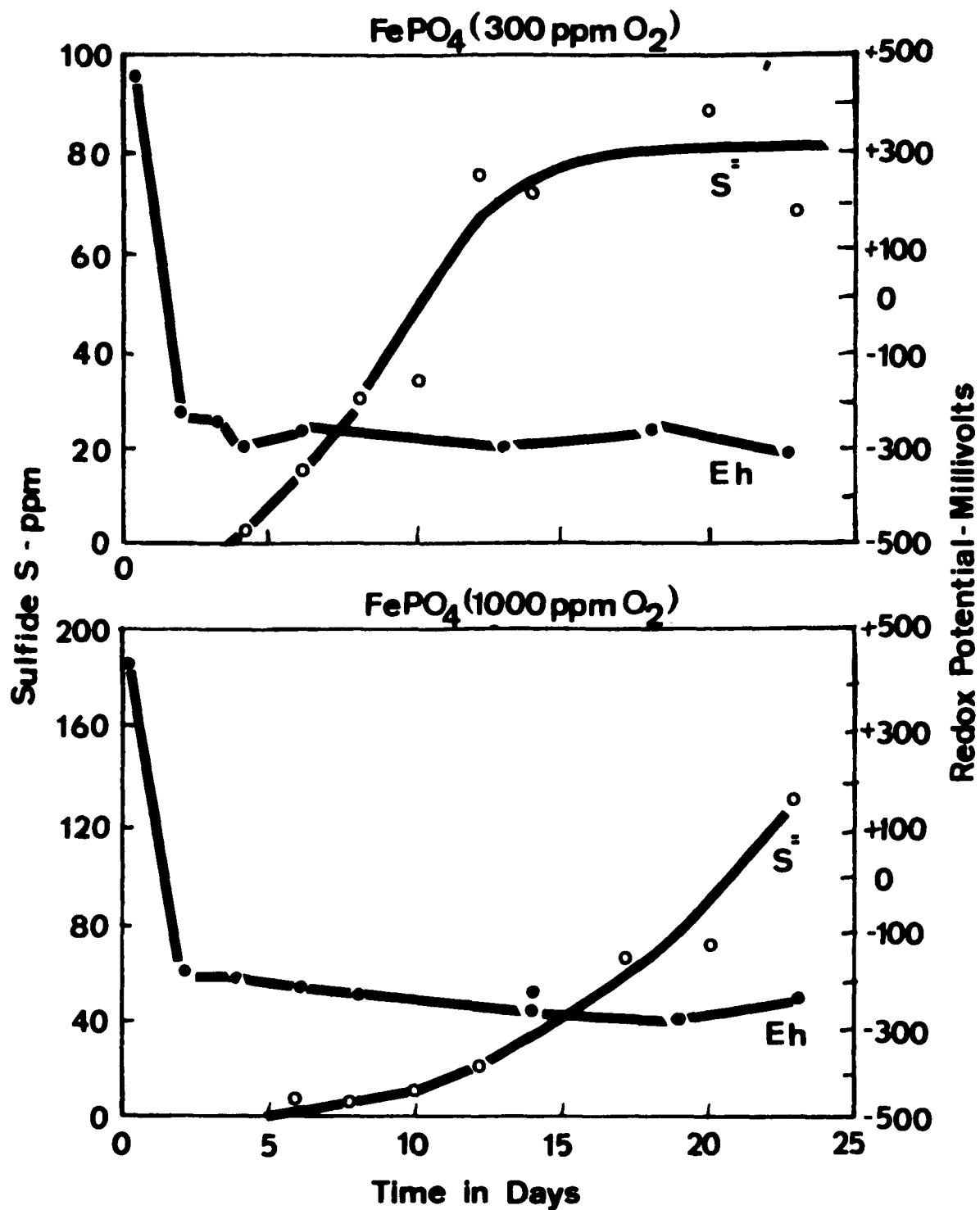


Figure 8. Sulfide produced from 100 and 200 ppm  $\text{SO}_4^{=}\text{-S}$  as affected by the addition of ferric phosphate, equivalent to 300 and 1000 ppm  $\text{O}_2$ , prior to flooding a Crowley silt loam. Redox potential values are also shown.

effective oxidant for reducing the maximum rate of sulfide formation. However, this oxidant did not significantly affect the initiation of sulfide formation. The results of this study indicated that the more soluble oxidizing compounds, potassium nitrate and ferricitrophosphate, had the greatest effect in delaying the initiation of sulfide formation. The potassium nitrate treatment equivalent to 1000 ppm  $O_2$  delayed sulfide production 17 days. The 1000 ppm  $O_2$  equivalent amount of ferricitrophosphate delayed sulfide evolution 12 days. The redox potential in each treated soil was buffered at a higher value than the nontreated soil. This was indicative of the presence of an oxidized compound. The more insoluble compounds such as manganese dioxide, ferric phosphate and ferric oxide had less effect in delaying sulfide production. On the other hand, the soluble compounds had less effect than the insoluble in reducing the rate of sulfide formation. This effect was probably due to the fact that the soluble oxidants were readily reduced during the initial portion of the study, consequently having little further effect in inhibiting sulfate reduction. This effect could be microbial in nature because the addition of the soluble highly oxidized compounds would offer a large quantity of easily reducible material to act as a terminal electron acceptor. Consequently,



the redox potential would remain elevated and reduction processes would not proceed until all or almost all of the easily reducible compounds had been reduced. Sulfate reduction would then proceed at a normal or near normal rate.

The more insoluble oxidants, namely manganese dioxide, ferric oxide and ferric phosphate, were not so readily reduced during the initial stages of reduction because of the stable nature of the oxidants. Thus, a reduction of the rate of sulfide formation by the more insoluble oxidizing agents was observed, while at the same time initial sulfide production was not noticeably delayed. This effect could be partially chemical in nature rather than a direct microbial effect. For the insoluble oxidants, the redox potential was sufficiently low for sulfide formation and initial evolution was not appreciably delayed; however, the maximum rate of sulfide formation was depressed. This suggests that as sulfide was microbially produced it was partially chemically oxidized by the oxidized compounds (manganese dioxide and ferric phosphate) that slowly become available (soluble).

Generally, the amount of ferric phosphate equivalent to 1000 ppm  $O_2$  had the greatest effect on the rate of sulfide formation. The rate of sulfide evolution was decreased by 24.1 ppm per day. Takijima et al. (1962)

reported that soils high in ferric iron exhibited very little sulfide toxicity. Ponnamperuma (1955) stated that soils abundant in iron would not exhibit sulfide toxicity. Turner and Patrick (1968) noted a buffering effect on the redox system of a flooded soil by the iron system that was considered sufficiently high to retard sulfate reduction. Manganese dioxide equivalent to 1000 ppm  $O_2$  also had a significant but slightly less pronounced effect on the maximum rate of sulfide formation. The rate of sulfide evolution was depressed by 16.7 ppm per day. Ponnamperuma et al. (1965) found that  $MnO_2$  added at a rate of 4000 ppm (equivalent to about 1067 ppm  $O_2$ ) in a field study with rice, corrected toxic conditions associated with a highly reduced soil. They also found the redox potential to be elevated above that of the untreated plot. Turner and Patrick (1968) demonstrated a buffering effect by the oxides of manganese on the redox potential of a flooded soil. They reported the redox potential to be greater than zero until manganous accumulation had reached a maximum.

The potassium nitrate treatment equivalent to 1000 ppm  $O_2$  had the greatest effect on delaying initial sulfide formation. The initiation of sulfide evolution was delayed 17 days. Ferricitrophosphate at the same amount delayed initial evolution of sulfide by 12 days.

Ferricitrophosphate was also effective in reducing the maximum rate of sulfide formation. Turner and Patrick (1968) demonstrated that as long as nitrate was present in a soil the redox potential was maintained sufficiently high to render the sulfate-reducers inactive. Yaun and Ponnamperuma (1966) reported that 4000 ppm nitrate (equivalent to about 2396 ppm  $O_2$ ) delayed further microbial reduction until all of the nitrate was reduced. Connell and Patrick (1969) found that as long as nitrate was present in a flooded soil no sulfate was reduced to sulfide.

The oxygen treatment was slightly effective in delaying the initiation of sulfide formation and reducing the maximum rate of sulfide evolution. The oxygen treatment had no prolonged effect as it was very actively reduced.

D. Sulfide Production as Affected by Various Oxidants  
Added to a Reduced Submerged Soil after Maximum  
Sulfide Formation

The purpose of this experiment was to determine the effects of adding various oxidants to a waterlogged Crowley silt loam after anaerobic reduction processes had progressed to a point where sulfide formation was a maximum. The capacity of these oxidants to oxidize sulfide was determined. The oxidants added to the

reduced soils were: oxygen, potassium nitrate, manganese dioxide, ferricitrophosphate and ferric phosphate. The results are presented in Figures 9 to 13.

The results of the control treatment shown in the upper part of Figure 9 demonstrate that no contamination from atmospheric oxygen occurred. The constant rate of sulfide formation of the control series show that no oxygen diffused through the serum cap of the sample bottles during the shaking and incubation period. It also showed that the atmosphere (argon) of the sample bottle was not contaminated with oxygen. This is demonstrated by the fact that the sulfide and redox potential curves remained relatively unchanged over the 72 hour study period.

The effect of adding 500 ppm oxygen on the sulfide content of the anaerobic soil is shown in the lower part of Figure 9. The data show an immediate and prolonged effect of oxygen. About two-thirds of the 183 ppm sulfide was rapidly oxidized by the oxygen. The sulfur had not been completely reduced to sulfide by the end of the 72 hour incubation period. The redox potential showed a marked and prolonged increase upon the addition of the oxygen and had not returned to the initial minimum value of -200 millivolts by the end of the experiment.

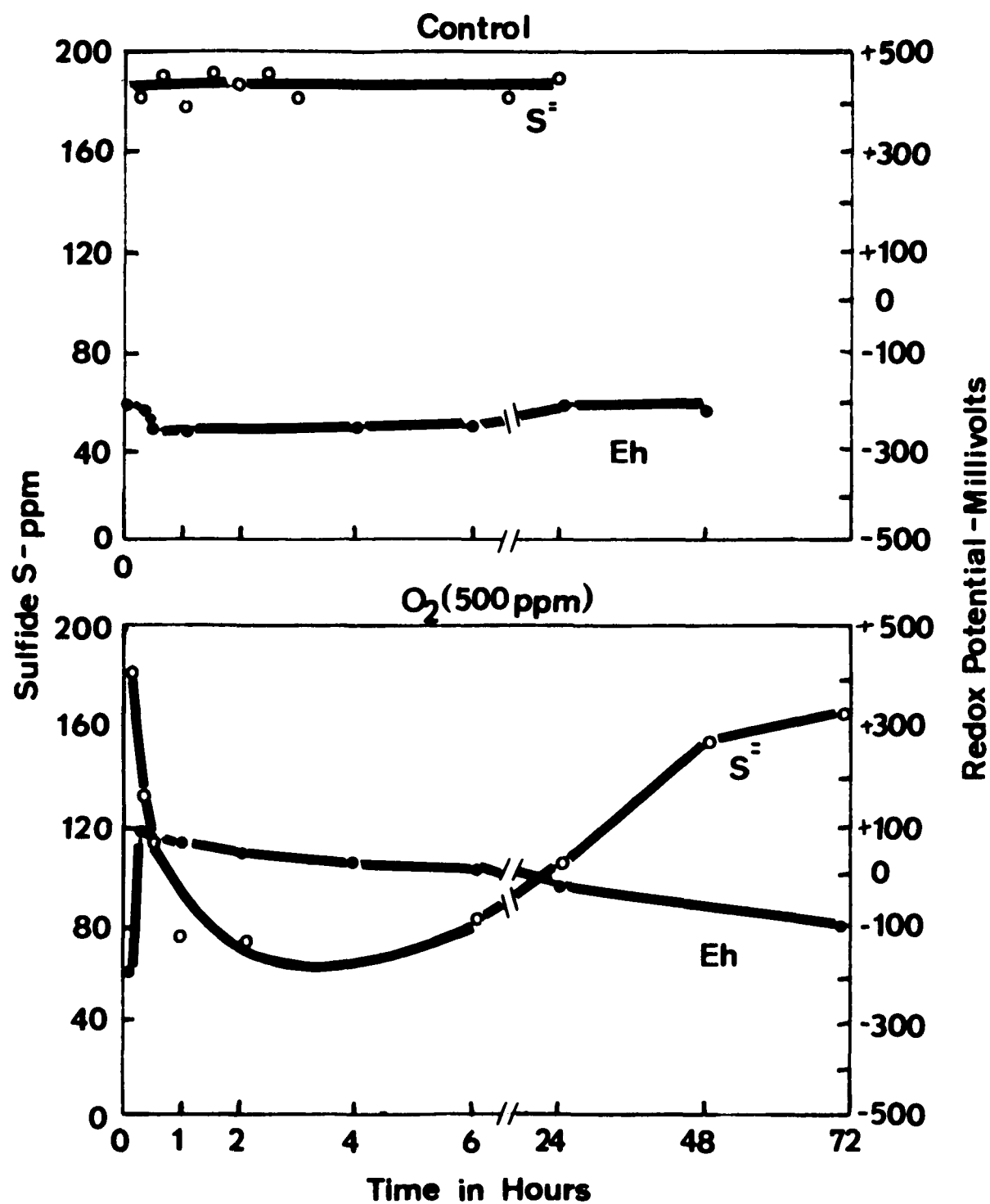


Figure 9. Maximum sulfide produced from 200 ppm  $\text{SO}_4^{2-}\text{-S}$  added prior to flooding a Crowley silt loam and sulfide produced from 200 ppm  $\text{SO}_4^{2-}\text{-S}$  as affected by the addition of 500 ppm  $\text{O}_2$  to a flooded Crowley silt loam after maximum sulfide formation. Redox potential values are also shown.

Figure 10 illustrates the effect of addition of two amounts of potassium nitrate on the sulfide content of a flooded soil. Potassium nitrate added at an amount equivalent to 1000 ppm  $O_2$  caused a sharp decrease in the total sulfide concentration and completely oxidized the sulfides with no sulfide reappearing for 22 hours. The oxidant equivalent to 500 ppm  $O_2$  had a much less pronounced effect and only oxidized about 60 ppm sulfide. The sulfide content of the soil receiving the low amount of oxidant had returned to its maximum value after about 6 hours. Sulfide formation of the soil that received the high concentration of oxidant was 53 ppm below the maximum level of 183 ppm at the termination of the 72 hour incubation period. The oxidants also caused the immediate oxidation of 20 and 50 ppm of the initial sulfide level as shown by the decrease in sulfide at zero time. The redox potential of the systems underwent a sharp and prolonged increase for both nitrate treatments with the highest concentration causing the greatest increase in potential. The redox potentials of both treatments had not decreased to the initial value by the end of the study.

The effect of the two amounts of manganese dioxide added to the reduced Crowley silt loam are found in Figure 11. Both of the manganese dioxide treatments

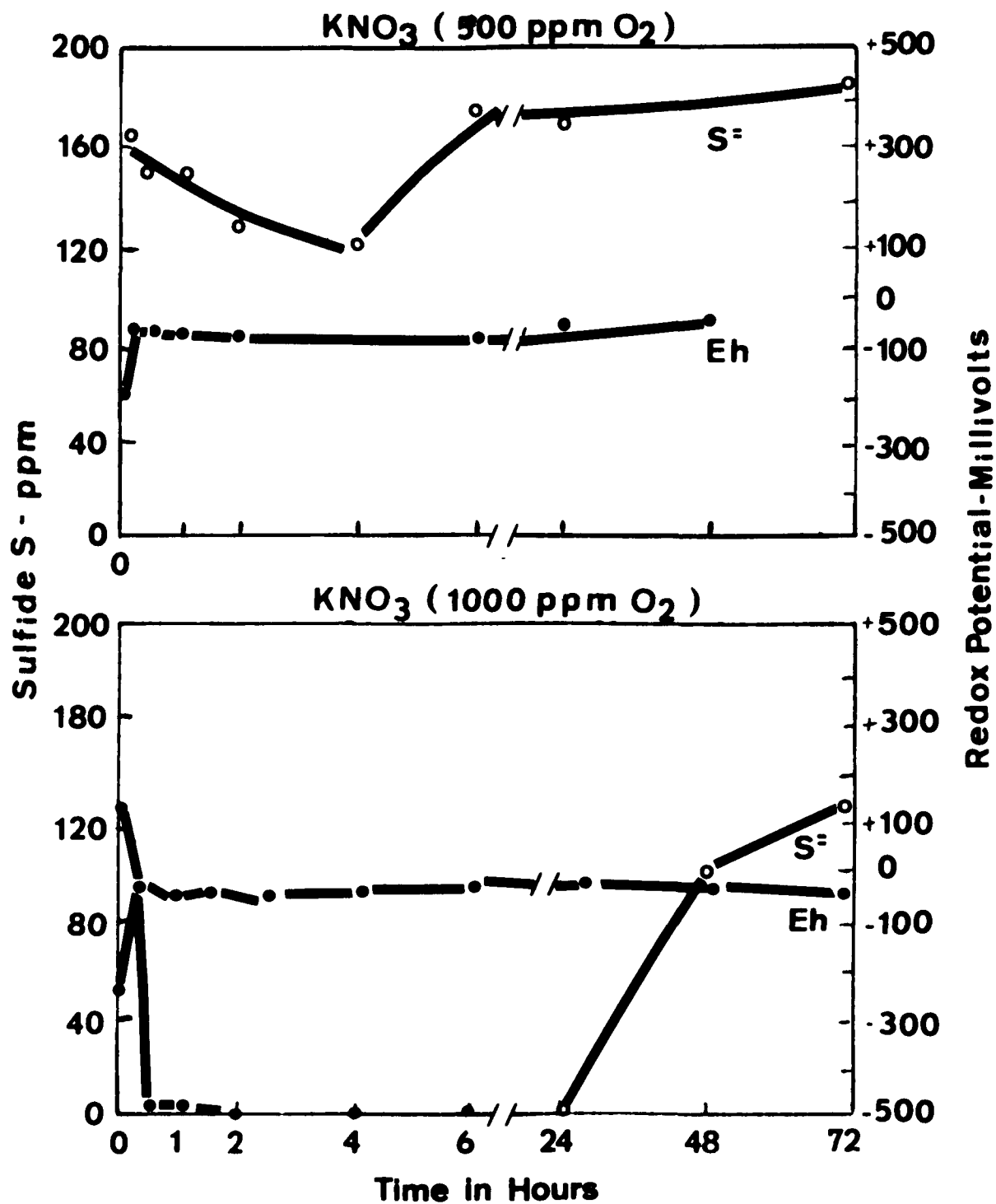


Figure 10. Sulfide produced from 200 ppm  $\text{SO}_4^{2-}$ -S as affected by the addition of potassium nitrate, equivalent to 500 and 1000 ppm  $\text{O}_2$ , to a flooded Crowley silt loam after maximum sulfide formation. Redox potential values are also shown.

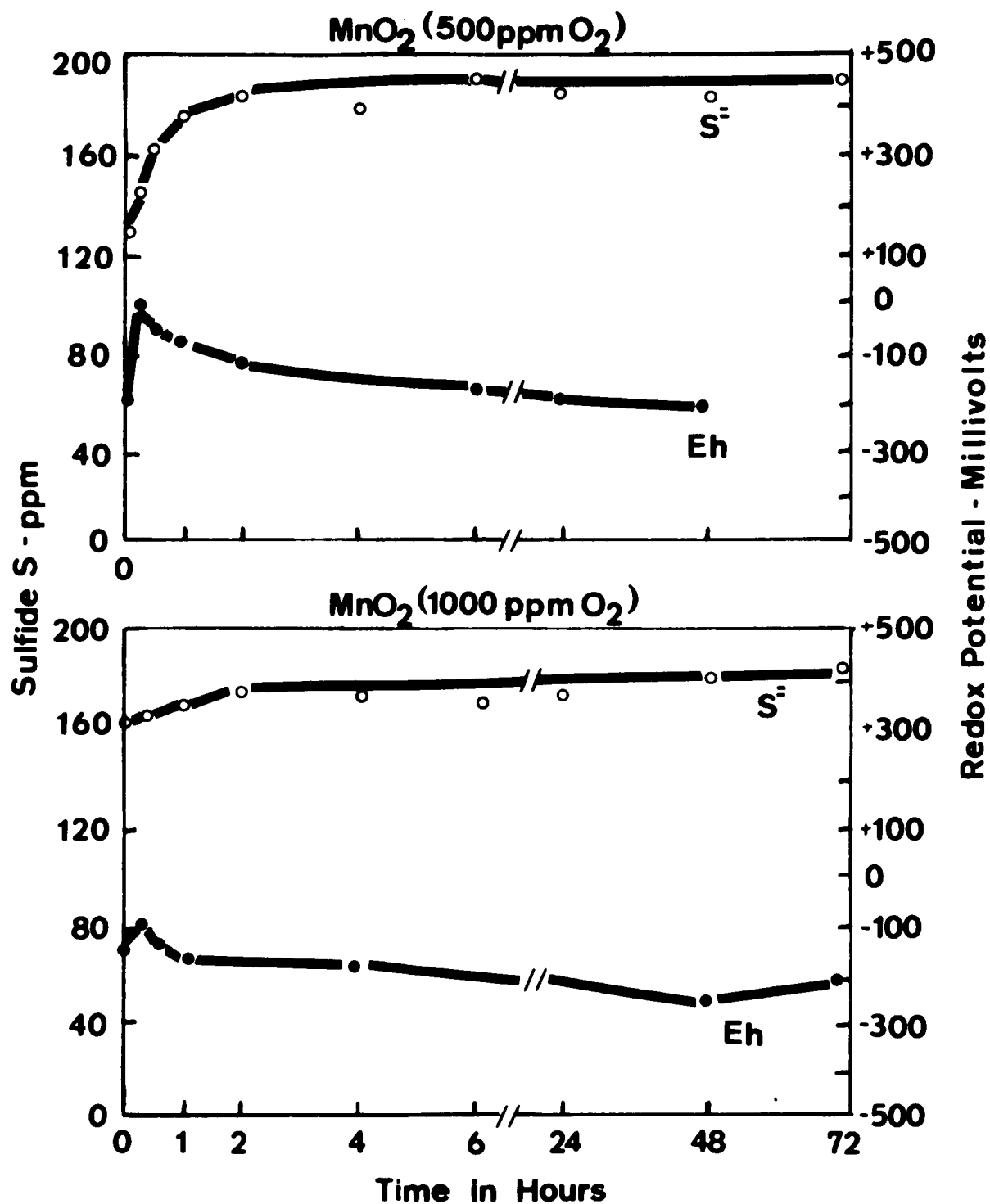


Figure 11. Sulfide produced from 200 ppm  $\text{SO}_4^{2-}\text{-S}$  as affected by the addition of manganese dioxide, equivalent to 500 and 1000 ppm  $\text{O}_2$ , to a flooded Crowley silt loam after maximum sulfide formation. Redox potential values are also shown.



caused a rapid though relatively small initial decrease in sulfide. The total sulfide concentration quickly returned to the initial value. This initial decrease may have occurred as a result of chemical oxidation during the acidification step in the sulfide analysis. Manganic manganese is a more powerful oxidizing agent under acidic conditions (Ponnamperuma et al., 1964). For samples incubated several hours the small amount of readily available manganic manganese added to the reduced soil was biologically reduced, resulting in less sulfide oxidation during the acidification step. The redox potential showed only a small rise as a result of addition of the manganese dioxide and quickly returned to about -200 millivolts.

The results obtained by the use of two concentrations of ferricitrophosphate to oxidize sulfides in a flooded soil are presented in Figure 12. That equivalent to 500 ppm  $O_2$  caused a very sharp 113 ppm decrease in the total sulfide concentration. Sulfide concentration returned to the initial content after about 24 hours. The redox potential of this treatment gave a corresponding increase with the addition of the oxidant and returned to the initial level of -200 millivolts after about 24 hours. Conversely, oxidant equivalent to 1000 ppm  $O_2$  appeared to overload the soil system, as 78 ppm sulfide was oxidized immediately upon the

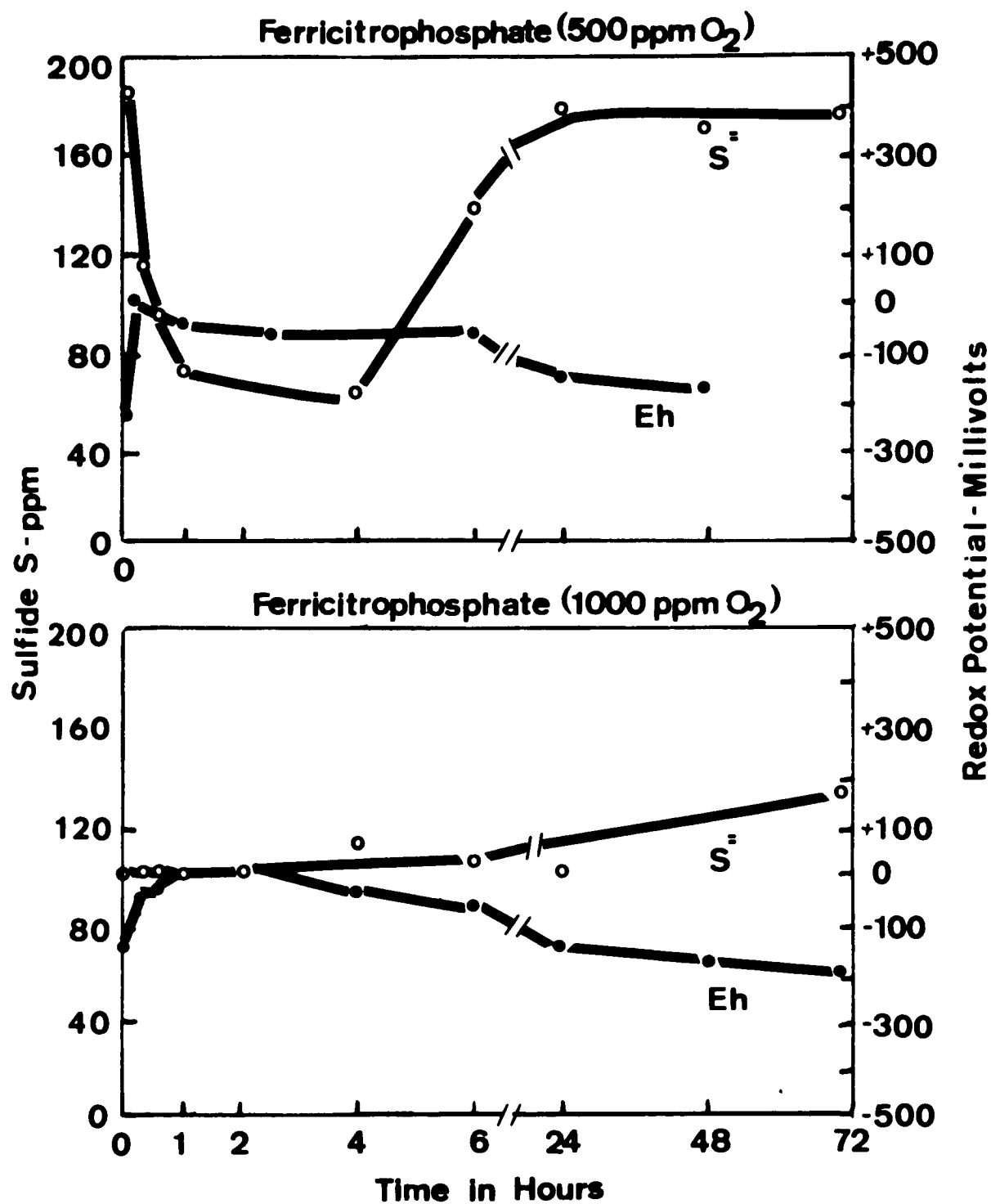


Figure 12. Sulfide produced from 200 ppm  $\text{SO}_4^{2-}\text{-S}$  as affected by the addition of ferricitrophosphate, equivalent to 500 and 1000 ppm  $\text{O}_2$ , to a flooded Crowley silt loam after maximum sulfide formation. Redox potential values are also shown.

addition of the oxidant. At the completion of the incubation period the total sulfide content of the soil receiving the high concentration of oxidant was about 30 ppm below the initial sulfide concentration of 183 ppm. However, after a sharp increase, the redox potential of the soil containing the high ferricitrophosphate decreased to the initial potential of -200 millivolts by the end of the study.

The data presented in Figure 13 illustrate the effects of the addition of ferric phosphate in amounts equivalent to 500 and 1000 ppm  $O_2$  on the sulfide content of a reduced flooded soil. Ferric phosphate equivalent to 500 ppm  $O_2$  caused a 43 ppm decrease in sulfide. Sulfide had reformed in the soil to the initial level of 183 ppm after 24 hours. The redox potential increased slightly with the addition of the oxidant and had returned to the initial level after 24 hours. The concentration of oxidant equivalent to 1000 ppm  $O_2$  resulted in a very sharp decrease of 100 ppm sulfide when added to the flooded soil. The sulfide content had not returned to the initial concentration of 183 ppm by the end of the 72 hour study. The redox potential gave a very sharp increase and remained elevated for 24 hours after the addition of the oxidant. The increase in potential caused by the higher rate of oxidant was higher and more prolonged than the potential change caused by the lower amount of oxidant.

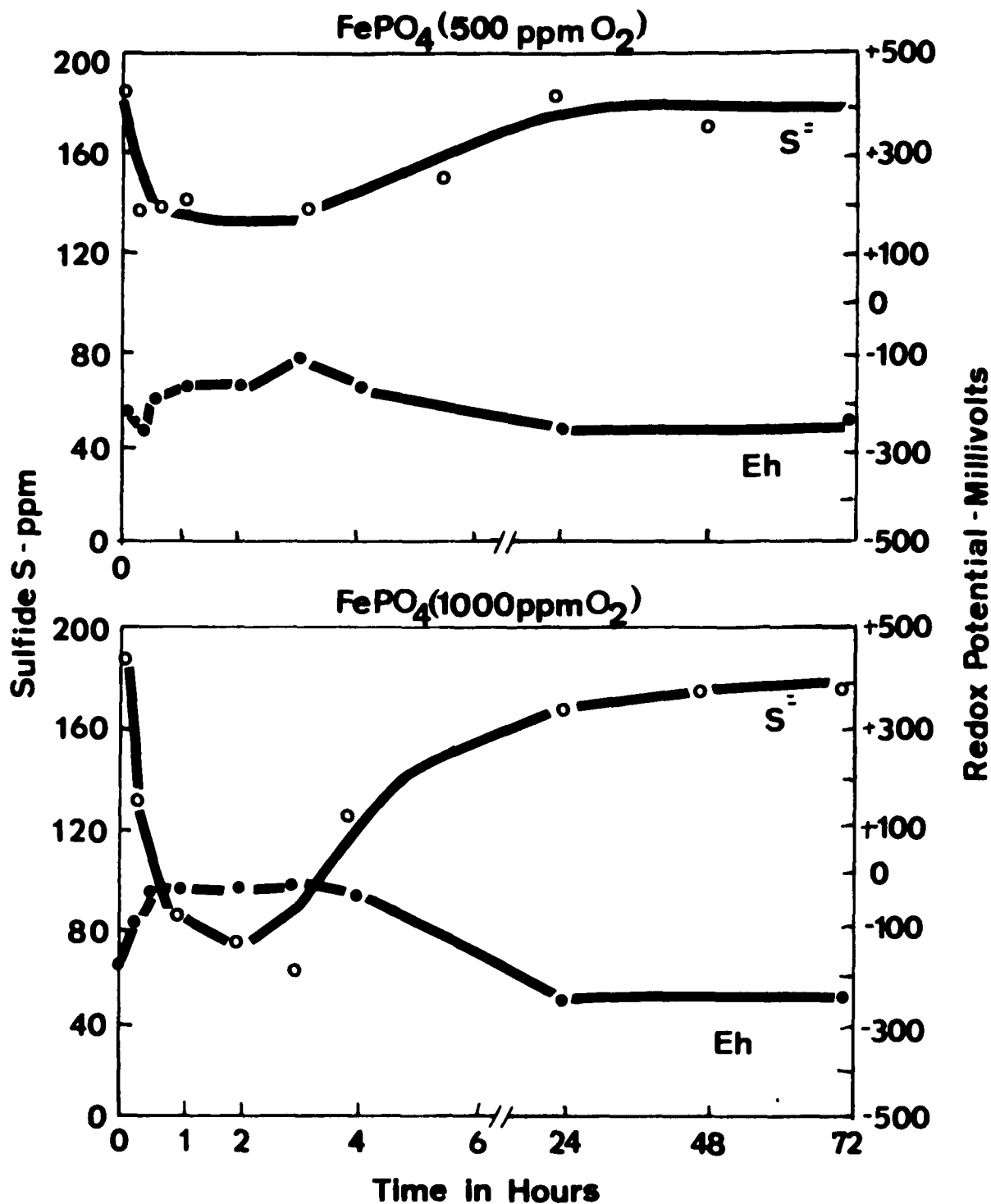


Figure 13. Sulfide produced from 200 ppm  $\text{SO}_4^{2-}$ -S as affected by the addition of ferric phosphate, equivalent to 500 and 1000 ppm  $\text{O}_2$ , to a flooded Crowley silt loam after maximum sulfide formation. Redox potential values are also shown.

Oxygen at an amount of 500 ppm appeared to be most effective in oxidizing sulfide. Over one-half of the sulfide was oxidized and the concentration of sulfide at the end of the study had not returned to the maximum content prior to adding the oxidant. Connell and Patrick (1969) demonstrated that a soil containing 120 ppm total sulfides decreased to 5 ppm when exposed to a constant oxygen supply and agitated for 2 hours. The potassium nitrate at a concentration equivalent to 1000 ppm  $O_2$  was the most effective sulfide oxidant at this higher rate. All of the sulfide was oxidized and several hours passed before sulfide formation resumed. Vamos (1958) stated that where he added nitrate to a flooded soil system the microbially produced atomic hydrogen was used to reduce the nitrate and in turn, could not be used by the sulfate-reducers as an energy source. Ponnampetuma (1955) found that applications of nitrate depressed the concentration of ferrous iron and organic reduction products and markedly improved the growth of rice in a flooded soil. Nitrate at a rate equivalent to about 2376 ppm  $O_2$  was found to retard sulfate reduction (Yaun and Ponnampetuma, 1966). Ferricitrophosphate was effective in oxidizing about one-half of the total sulfide when added equivalent to 500 or 1000 ppm  $O_2$  and resulted in the most prolonged sulfide oxidation for the oxidants added at a rate

equivalent to 1000 ppm  $O_2$ . About one-third of the sulfide was oxidized by the ferricitrophosphate at the completion of the incubation period, resulting in the lowest final concentration of sulfide for all treatments. Takijima et al. (1962) stated that Japanese soils high in iron produce very little  $H_2S$  and the soluble sulfide content is insignificant. Bloomfield (1969) added synthesized  $Fe_2O_3$  in a slight excess of Fe over the  $SO_4^{-2}$  equivalent to prevent free sulfide accumulation. Although less soluble than the previously mentioned oxidants, the ferric phosphate treatments were intermediate in the oxidation of total sulfide. The low concentration oxidized about one-fourth of the sulfide and the rate equivalent to 1000 ppm  $O_2$  oxidized slightly over one-half of the soil sulfide. Neither had a prolonged effect on sulfide. The insoluble manganese dioxide treatments were ineffective in oxidizing sulfides present in the anaerobic soil. This oxidant when mixed with a soil prior to flooding was effective in buffering the redox potential at a more positive value and reducing the maximum rate of sulfide formation. However, manganese dioxide was ineffective as a sulfide oxidant.

Generally the soluble oxidants (potassium nitrate and ferricitrophosphate), when added to a soil prior to flooding, were effective in delaying the initiation

of sulfide formation and were equally effective in decreasing the content of accumulated sulfides when added to a reduced flooded soil. However, the soluble oxidants did not significantly decrease the maximum rate of sulfide production when added prior to flooding. On the other hand, the insoluble oxidants were only partially effective (ferric phosphate) and ineffective (manganese dioxide) in decreasing the concentration of accumulated sulfides when added to a reduced flooded soil, and were ineffective in delaying the initiation of sulfide formation when mixed with a soil prior to flooding. However, the insoluble oxidants were very effective in reducing the maximum rate of sulfide production when added to a soil prior to submergence.

E. Heavy Metal Sulfide Reactions in Aerobic and Anaerobic Soils

A study was conducted to evaluate the effects of aerobic (optimum moisture or 1/3 bar moisture tension) and anaerobic (flooded) soil moisture conditions on the oxidation and subsequent solution of sulfur from heavy metal sulfides mixed with the soil. The sulfide salt of most metals is usually less soluble than the sulfate salt. The isotope  $^{35}\text{S}$  was utilized to study the stability of sulfide-sulfur originating from the metal sulfide placed in the aerobic or anaerobic soil.

Several metal sulfide compounds were synthesized in the laboratory by incorporating  $^{35}\text{S}$  into the salt. The metal sulfide salts synthesized were, manganous sulfide ( $\text{MnS}$ ;  $K_{\text{sp}} = 1.4 \times 10^{-15}$ ), ferrous sulfide ( $\text{FeS}$ ;  $K_{\text{sp}} = 3.3 \times 10^{-19}$ ), zinc sulfide ( $\text{ZnS}$ ;  $K_{\text{sp}} = 1.2 \times 10^{-23}$ ), copper sulfide ( $\text{CuS}$ ;  $K_{\text{sp}} = 3.5 \times 10^{-38}$ ), and mercuric sulfide ( $\text{HgS}$ ;  $K_{\text{sp}} = 1 \times 10^{-50}$ ). The aerobic soil was considered to be that in which the soil moisture was kept at optimum field moisture (1/3 bar moisture tension), thus allowing the large soil pores to be free of water and be open to oxygen movement. An anaerobic environment was developed by flooding the soil with water, resulting in restricted oxygen movement into the soil.

The oxidation of the insoluble sulfide salts was traced by a water extract for the soluble oxidized forms of sulfur arising from the radioactive sulfide. The data giving evidence of the oxidation of the metal sulfides are presented in Figures 14 to 18. The water-extractable  $^{35}\text{S}$  is presented as counts per minute per gram of soil and also as percent recovery of added  $^{35}\text{S}$ . The oxidation of the sulfide to the water-soluble oxidized forms ( $\text{SO}_4^{=}$ ,  $\text{SO}_3^{=}$ ,  $\text{S}_2\text{O}_3^{=}$ ,  $\text{S}_4\text{O}_6^{=}$ ) and a subsequent radioassay for  $^{35}\text{S}$  should give a clear evaluation of the transformations, because the water extract removed only the oxidized sulfur forms and left the insoluble



sulfide salts. The solubility product constants (Garrels and Christ, 1965; Krauskopf, 1967) for each sulfide salt are also given in the figures. An increase in extractable radioactive sulfur indicated oxidation of the metal sulfide either chemically or microbiologically. Consequently, this oxidative process enhances recycling of the relatively stable metal sulfide into other more soluble forms in the soil environment.

The data displayed in Figure 14 show the effect of an aerobic soil as compared to an anaerobic soil on the transformation undergone by manganous sulfide. Manganous sulfide with a solubility product constant of  $1.4 \times 10^{-15}$  was the most soluble of the metal sulfide salts studied. The labelled manganous sulfide was added to the soil at a rate of 8750 cpm  $^{35}\text{S}$  per gram of soil. The data in Figure 14, presented as counts per minute of water extractable  $^{35}\text{S}$  per gram of soil and percent recovery of added  $^{35}\text{S}$ , demonstrate that manganous sulfide remained stable under flooded conditions. Under aerobic conditions, however, the sulfide salt was readily oxidized, resulting in an increase in water-extractable  $^{35}\text{S}$ . About 50% of the  $^{35}\text{S}$  labelled manganese sulfide was recovered as water-extractable  $^{35}\text{S}$ . However, about 5% of the added  $^{35}\text{S}$  was recovered at zero time which resulted in a net recovery of about 45% of the  $^{35}\text{S}$  from the aerobic soil.

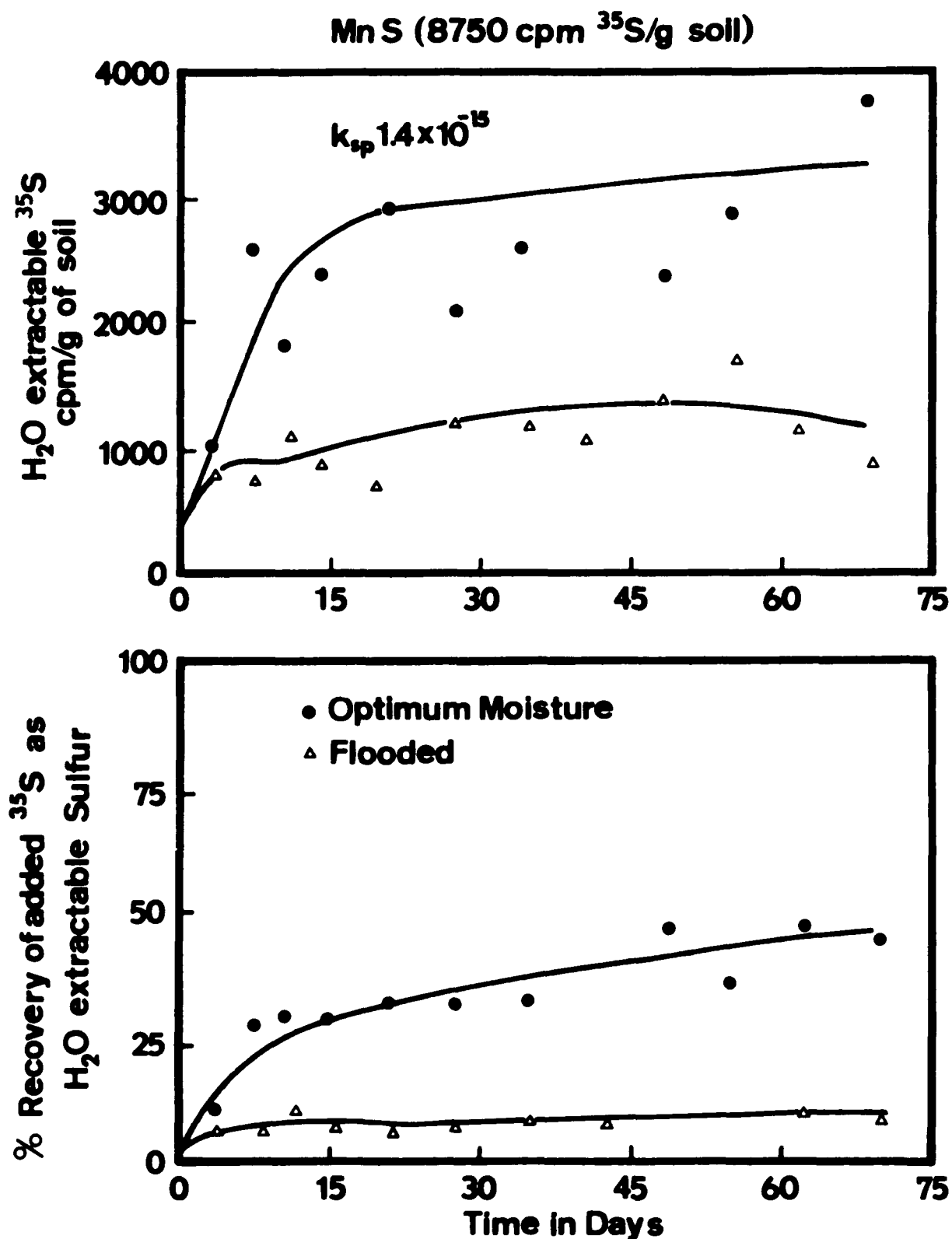


Figure 14. The effects of aerobic (optimum moisture) and anaerobic (flooded) soil systems on the water extractable  $^{35}\text{S}$  originating from  $^{35}\text{S}$  labelled manganese sulfide added to a Crowley silt loam.

The recovery of the  $^{35}\text{S}$  immediately after mixing with the soil suggests that a small portion of the sulfide was oxidized during the soil labelling procedure.

Figure 15 depicts the effects of the aerobic and anaerobic soil systems on the stability of  $^{35}\text{S}$  labelled ferrous sulfide. The radioactively labelled FeS was added to the soil at a rate of 12,000 counts per minute of  $^{35}\text{S}$  per gram of soil. The FeS was stable in the flooded soil. About 60% of the radioactively labelled FeS was recovered from the aerobic soil as water-extractable  $^{35}\text{S}$ . After subtracting the amount of extractable  $^{35}\text{S}$  found at zero time from the maximum extractable  $^{35}\text{S}$  the net percent recovery of  $^{35}\text{S}$  was about 50%. Although the ferrous sulfide was theoretically more insoluble ( $K_{\text{sp}} = 3.7 \times 10^{-19}$ ) than the MnS, more water-extractable  $^{35}\text{S}$  was released under the aerobic soil conditions. This could be due to the MnS being less amorphous than the FeS; a less amorphous compound would in most cases be more insoluble due to decreased surface area.

Data in Figure 16 show the effects of an aerobic and anaerobic soil on the solubility of labelled zinc sulfide added to the soils. The  $^{35}\text{S}$  labelled salt was added to the soil at a rate of 12,275 cpm per gram of soil. Zinc sulfide appeared to be very reactive during the first five days after flooding as noted by

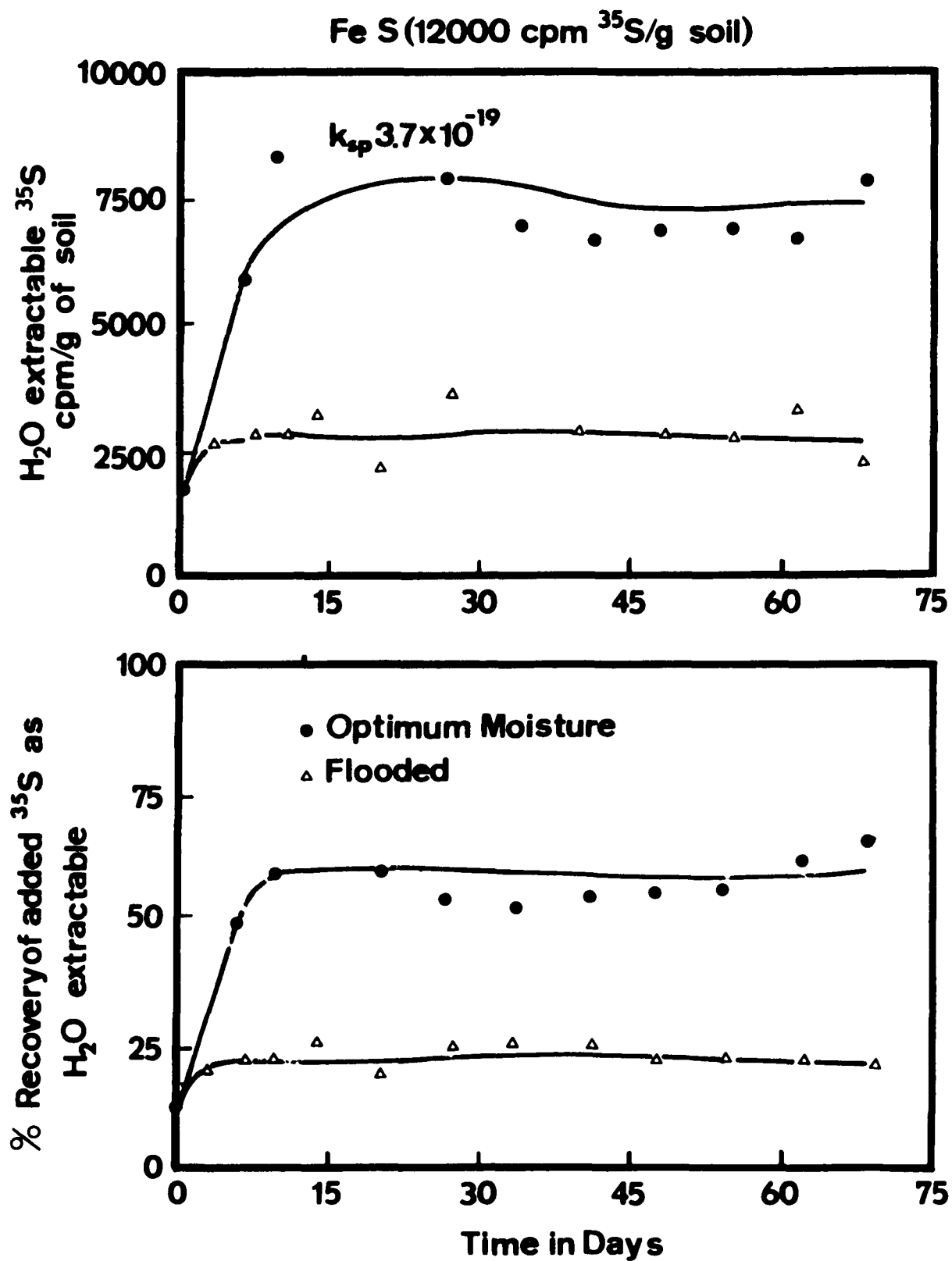


Figure 15. The effects of aerobic (optimum moisture) and an-aerobic (flooded) soil systems on the water extractable  $^{35}\text{S}$  originating from  $^{35}\text{S}$  labelled ferrous sulfide added to a Crowley silt loam.

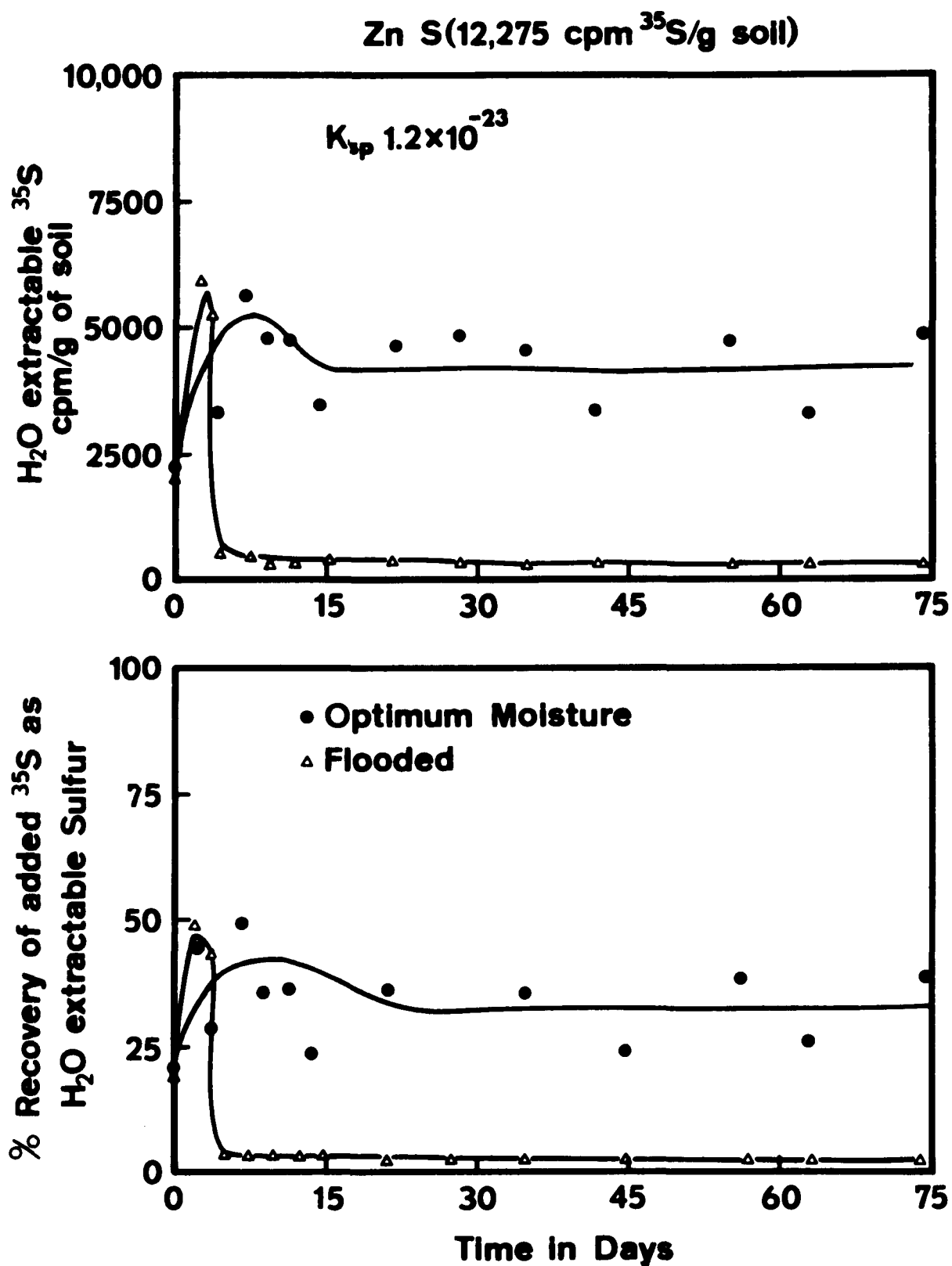


Figure 16. The effects of aerobic (optimum moisture) and anaerobic (flooded) soil system on the water extractable  $^{35}\text{S}$  originating from  $^{35}\text{S}$  labelled zinc sulfide added to a Crowley silt loam.

the increase of water-extractable  $^{35}\text{S}$ . After the fifth day of incubation strong reducing conditions had set in, apparently resulting in additional sulfide being produced and subsequent stabilization of the zinc sulfide by reprecipitation of the  $^{35}\text{S}$ . After the fifth day of incubation the zinc sulfide appeared very stable under the flooded conditions with very little of it decomposing as demonstrated by the low amount of water-extractable  $^{35}\text{S}$ . Under aerobic soil conditions zinc sulfide appeared slightly more stable than did MnS or FeS with less than 40% recovery of the added  $^{35}\text{S}$  as water-extractable sulfur. However, about 20% of the added  $^{35}\text{S}$  was recovered at zero time which would give a net  $^{35}\text{S}$  recovery of about 20%. It was also noted that the concentration of water-extractable  $^{35}\text{S}$  appeared slightly erratic during the first week of incubation under aerobic conditions.

Figure 17 depicts the solubility of  $^{35}\text{S}$  labelled copper sulfide added to an aerobic and anaerobic soil. The labelled copper sulfide was added at a rate of 47,500 cpm  $^{35}\text{S}$  per gram of soil. The decomposition of the salt is given as cpm water-extractable  $^{35}\text{S}$  per gram of soil, and as percent recovery of added  $^{35}\text{S}$ . Copper sulfide ( $K_{\text{sp}} = 3.5 \times 10^{-38}$ ), although more insoluble than the previously reported metal sulfides, released about 35% of the  $^{35}\text{S}$  as water-extractable sulfur under

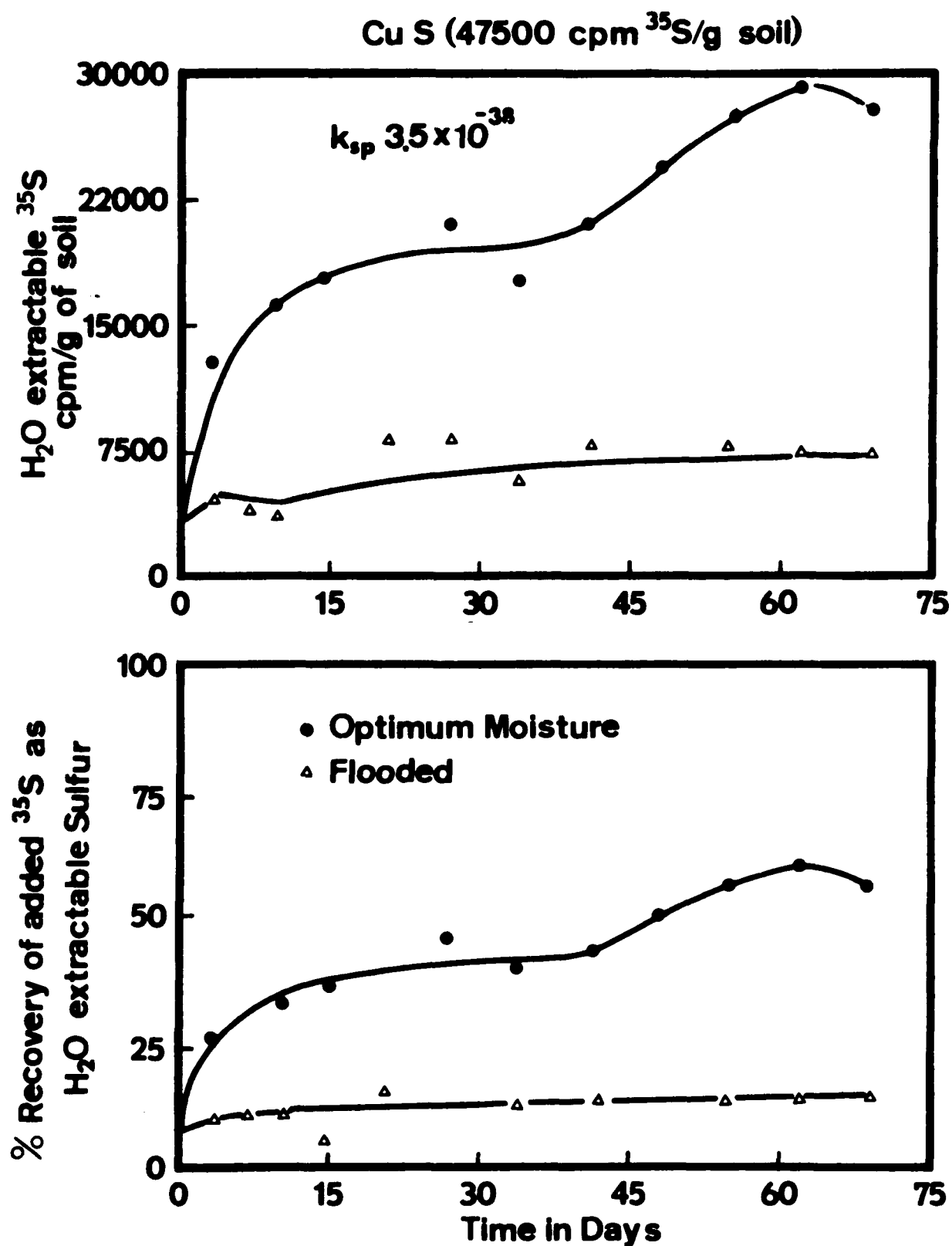


Figure 17. The effects of aerobic (optimum moisture) and anaerobic (flooded) soil systems on the water extractable  $^{35}\text{S}$  originating from  $^{35}\text{S}$  labelled copper sulfide added to a Crowley silt loam.

aerobic conditon during the first 40 days of incubation. During the last 20 days of incubation under aerobic conditions, the insoluble CuS released 55% of the added  $^{35}\text{S}$  as water-extractable sulfur. After correcting for the initial 10% recovery of  $^{35}\text{S}$ , the net recovery of  $^{35}\text{S}$  from the aerobic soil was 25% and 45% for the first two-thirds and last one-third of the incubation period. No explanation for this increase in water-extractable  $^{35}\text{S}$  during the last stage of incubation can be given. Under anaerobic conditions, the copper sulfide appeared very stable. Less water-extractable  $^{35}\text{S}$  was obtained under the flooded conditions from copper sulfide than was obtained from manganous sulfide or ferrous sulfide, demonstrating the insoluble nature of the compound under anaerobic conditions. However, more  $^{35}\text{S}$  was extracted from manganese sulfide than from zinc sulfide. It appears that the reported solubility product constant was determined for a more crystalline form than may have been synthesized for this study.

The effects of aerobic and anaerobic soil conditions on the possible oxidation and dissolution of mercuric sulfide ( $\text{HgS}$ ;  $K_{sp} = 1 \times 10^{-50}$ ) are shown in Figure 18. The mercury sulfide was mixed with the soil at a rate of 2,500 cpm  $^{35}\text{S}$  per gram of soil and the subsequent decomposition given as cpm water-extractable



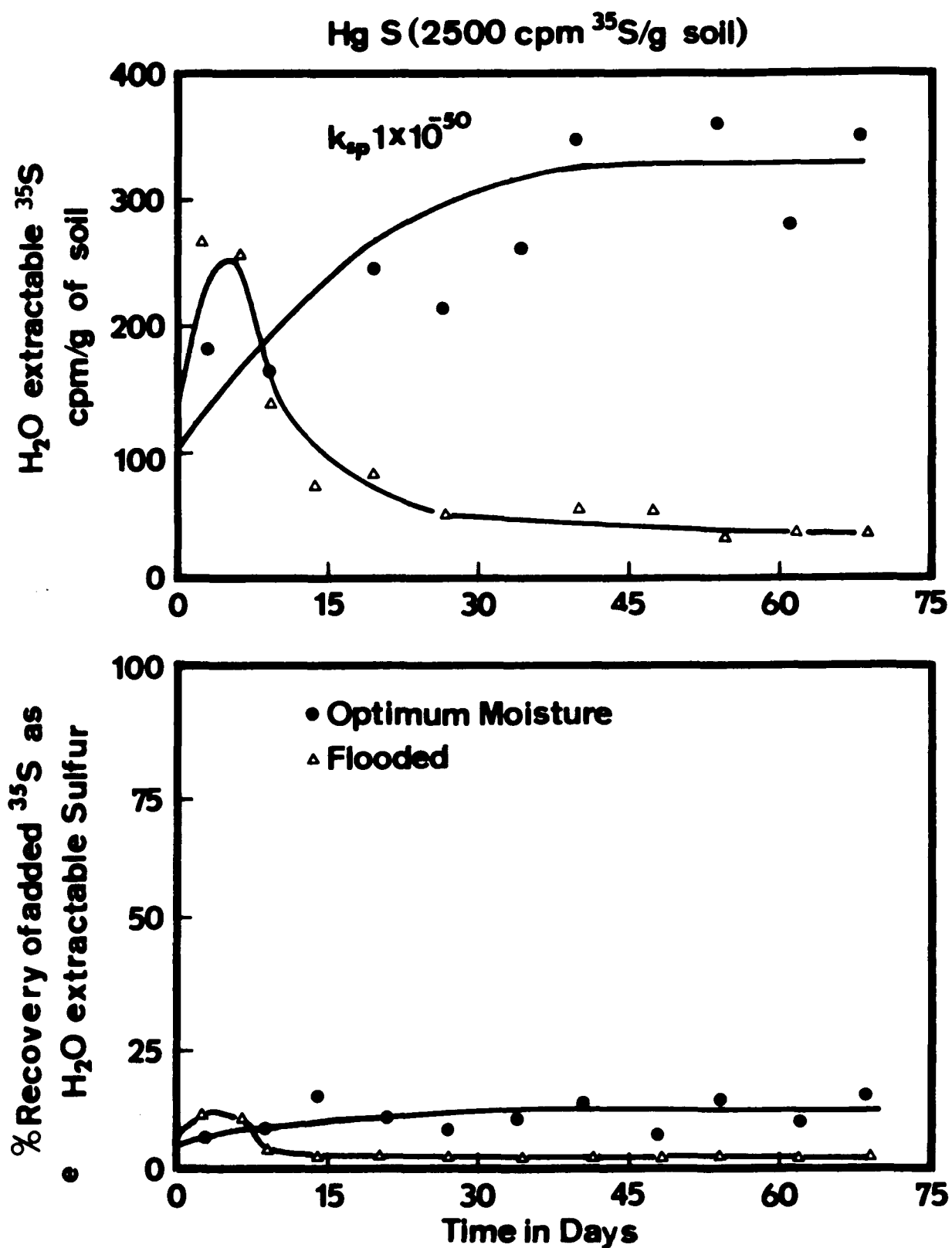


Figure 18. The effects of aerobic (optimum moisture) and anaerobic (flooded) soil systems on the water extractable  $^{35}\text{S}$  originating from  $^{35}\text{S}$  labelled mercury sulfide added to a Crowley silt loam.

$^{35}\text{S}$  per gram of soil and percent recovery of added  $^{35}\text{S}$ . Under flooded conditions,  $\text{HgS}$  was quite stable with only a 3% recovery of the added  $^{35}\text{S}$ . As occurred in varying degrees with the other metal sulfide treatments, an increase in water-extractable  $^{35}\text{S}$  was noted during the first few days after flooding. When intense reduction had set in, very little water-extractable  $^{35}\text{S}$  was found. Under aerobic soil conditions, only about 12% of the added  $^{35}\text{S}$  in mercury sulfide was recovered as water-extractable sulfur. After correcting for extractable  $^{35}\text{S}$  found at zero time, the net recovery of water-extractable  $^{35}\text{S}$  was found to be about 7%. This was considerably less than that found for  $\text{MnS}$ ,  $\text{FeS}$ ,  $\text{ZnS}$ , or  $\text{CuS}$  salts. A 7% dissolution of a very stable salt under aerobic soil conditions is particularly important in light of the current rate (Wallace et al., 1971) of mercury pollution of water covered sediments. There are certainly situations where these polluted sediments may be aerated (Geological Survey Staff, 1970) and subsequently oxidized and the mercury sulfide salts readily formed under anaerobic conditions are recycled into the environment. The decomposition of a relatively stable compound containing an element with the toxicity of mercury would intensify its toxic effect.

The results of this study indicate that under anaerobic soil conditions the metal sulfides remained very stable with little or no dissolution or separation of the  $^{35}\text{S}$  from the metal. This conclusion was reached because of no appreciable increase in the water-extractable  $^{35}\text{S}$  from the flooded soil at the completion of the incubation period. The major difference noted among the sulfide salts was that relatively less water-extractable  $^{35}\text{S}$  was obtained under both anaerobic and aerobic conditions as the solubility of the metal sulfides decreased. It should be noted that under the anaerobic conditions, especially at the initial phase of the study, elevated concentrations of water-extractable  $^{35}\text{S}$  were obtained but these amounts decreased as intense reducing conditions set in. During the first few days after flooding zinc sulfide and mercury sulfide salts gave a sharp initial increase and then a subsequent sharp decrease in the amount of water-extractable  $^{35}\text{S}$  recovered from the labelled salt. Manganous sulfide, ferrous sulfide, and copper sulfide gave a slight increase upon flooding and remained stable. Initially after flooding a soil, reducing conditions are not intense and with respect to very reduced compounds, the soil conditions may be oxidizing for some time even though only a small amount of free oxygen may be present. Subsequently, the sulfide

salts could be partially oxidized resulting in an increase in water-extractable  $^{35}\text{S}$ . However, the initial increase of water-extractable  $^{35}\text{S}$  may be due to the greater solution to solute (metal sulfide) ratio which may cause a larger amount of the  $^{35}\text{S}$  to come into solution. When intense reducing conditions prevail the  $^{35}\text{S}$  may then be reprecipitated or complexed into insoluble salts. The  $\text{ZnS}$  and  $\text{HgS}$  salts were more pronounced in this characteristic than  $\text{MnS}$ ,  $\text{FeS}$  and  $\text{CuS}$ .

Under aerobic soil conditions, the extractable  $^{35}\text{S}$  generally decreased with a decrease in solubility of the sulfide salts. The net percent recovery of  $^{35}\text{S}$  from manganous sulfide, ferrous sulfide, zinc sulfide, copper sulfide, and mercury sulfide were 45, 50, 20, 25, and 7%, respectively. As evidenced by the data, the decrease in extractable  $^{35}\text{S}$  only partially followed the pattern set by the solubility products of the respective sulfide salts. The basic reason for this may be that the solubility of the synthesized sulfide salts may have varied in some degree from the solubility products reported in the literature and also there may have been some variation among the synthesized salts in the degree of crystallinity. The cited solubility products were determined for the more crystalline salts formed in nature by geological evolution (Garrels and Christ, 1965). Consequently, the

synthesized salts may more closely resemble the more amorphous salts that are formed in flooded soils and sediments. However, of greater importance is that very stable metal sulfides such as mercury sulfide, copper sulfide, and zinc sulfide are recycled in aerated soil environments. The oxidation of copper and zinc sulfide is very important in the micronutrient nutrition of plants growing on these soils. In contrast, an oxidation and dissolution of a stable compound containing mercury is important to all forms of life in the biological food chain.

#### F. Reactions of Mercury in Aerobic and Anaerobic Soils

Increasing environmental pollution has placed special emphasis in the past two years on mercury reactions in marsh, lake, and river sediments. The chemistry of a flooded soil is very similar to that of a flooded sediment. Microbial genera, pH, oxidation-reduction reactions, organic components, and mineral constituents of flooded soils are similar to that of many sediments. The preceding study placed emphasis on iron, manganese, zinc, copper, and mercury sulfide transformations in aerobic and anaerobic soils. The isotope  $^{35}\text{S}$  was used to study these reactions. The following experiments take into consideration the oxidation of mercury sulfide and other reactions of Hg

in aerobic, anaerobic, and alternately aerobic and anaerobic soils. The isotope  $^{203}\text{Hg}$  was used to study these transformations.

Mercury sulfide was synthesized and labelled with  $^{203}\text{Hg}$  and mixed with a Crowley silt loam. The mercury sulfide was mixed with the soil to give a radioactivity of 1032 cpm  $^{203}\text{Hg}$  per gram of soil. To study the oxidation of the salt under two conditions, a subsample of the labelled soil was flooded continuously for the 70 day incubation period, while another subsample was kept aerobic (1/3 bar moisture tension) for 40 days and then flooded for the remainder of the 70 day study. Dissolution of mercury sulfide was measured by a water extract of the soil, and then radioactivity of the extract was indicative of the decomposition of the sulfide salt. The results of these two studies are presented in Figure 19. The oxidation or dissolution of the mercury sulfide is given as counts per minute of water-extractable  $^{203}\text{Hg}$  per gram of soil.

The results of the first portion of the experiment in which the soil was continuously flooded demonstrate that the sulfide salt was stable under anaerobic conditions. Only about 1% of the added  $^{203}\text{Hg}$  was recovered as water-soluble  $^{203}\text{Hg}$ . A slight, but insignificant increase in extractable  $^{203}\text{Hg}$  was noted by the end of the study. In comparison, the results of the preceeding

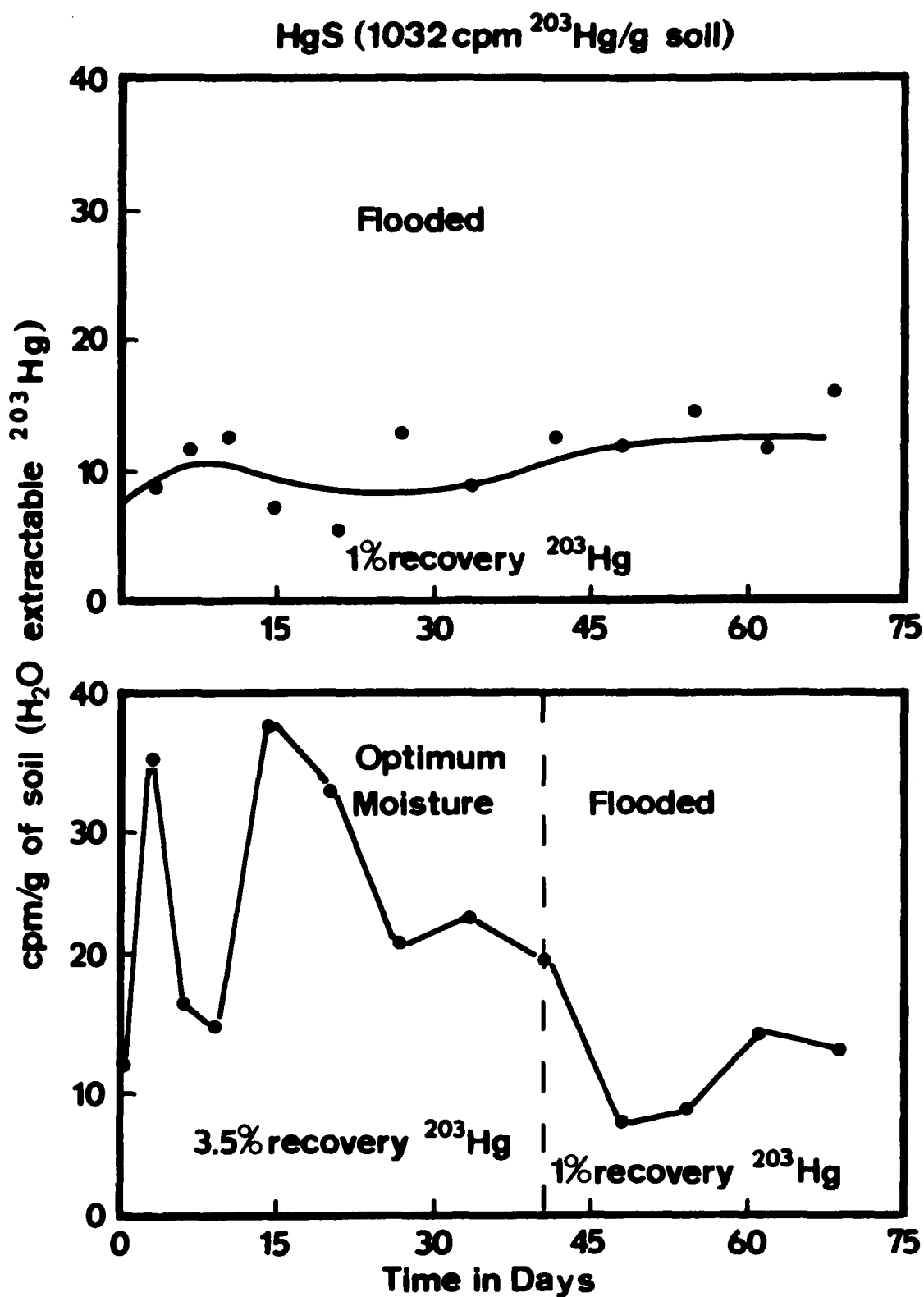


Figure 19. The effects of an anaerobic (flooded) soil system and an alternate aerobic (optimum moisture) and anaerobic soil system on the water extractable  $^{203}\text{Hg}$  originating from  $^{203}\text{Hg}$  labelled mercury sulfide added to a Crowley silt loam.

study in which  $^{35}\text{S}$  labelled mercury sulfide was incubated in a continuously flooded soil show about a 3% recovery of the added  $^{35}\text{S}$ . The three-fold decrease in recovery of  $^{203}\text{Hg}$  from the sulfide salt may be due to the adsorption of ionic mercury onto the soil cation exchange complex. The lower graph of Figure 19 depicts the effect of an alternate aerobic and anaerobic soil environment on the dissolution of mercury sulfide. A water extract for  $^{203}\text{Hg}$  was used to measure this transformation. Under the aerobic condition, an average of about 3.5% of the added  $^{203}\text{Hg}$  was recovered. Although the recovery data were erratic, the average recovery data appear to agree favorably with those obtained from the  $^{35}\text{S}$  labelled mercury sulfide. However, the recovery of  $^{203}\text{Hg}$  was slightly less than the recovery of  $^{35}\text{S}$  from the  $\text{Hg}^{35}\text{S}$  added to the aerobic soil. This, again, suggests that the ionic mercury is adsorbed onto the soil cation exchange complex. Upon flooding, the water-extractable  $^{203}\text{Hg}$  decreased to about 1% recovery of the added  $^{203}\text{Hg}$ . The three-fold decrease in water-extractable  $^{203}\text{Hg}$  indicates that mercury sulfide may be more stable under flooded conditions. The lower recovery of  $^{203}\text{Hg}$  as contrasted to  $^{35}\text{S}$  recovered under flooded conditions, also suggests that a portion of the soluble  $^{203}\text{Hg}$  was adsorbed by the soil cation exchange mechanism. Because of the capability of mercury



to sorb readily on a variety of silicate and organic materials (Geological Survey Staff, 1970), using water-extractable mercury as a measure of the dissolution of an insoluble mercury salt may underestimate the actual reaction rate. However, the recovery of  $^{203}\text{Hg}$  from  $^{203}\text{HgS}$  followed the same general pattern that was obtained by the recovery of  $^{35}\text{S}$  from  $\text{Hg}^{35}\text{S}$  incubated under identical conditions.

The preceeding experiments emphasized transformations undergone by very insoluble mercury sulfide. Another experiment was devised to evaluate the extractability of very soluble mercuric chloride mixed with a soil which was continuously flooded. The isotope  $^{203}\text{Hg}$  as  $^{203}\text{HgCl}_2$  was used to measure the relative changes of extractable mercury in the anaerobic soil. The  $^{203}\text{Hg}$  was added to the soil at a relative rate of 5142 cpm per gram of Crowley silt loam. Normal ammonium acetate was used to extract the adsorbed mercury. A rigorous 6 hour soil extraction procedure with several successive extractions was used. The results of this study are presented in Figure 20. The relative change in extractable mercury is given as cpm ammonium acetate-extractable  $^{203}\text{Hg}$  per gram of soil and as percent recovery of added  $^{203}\text{Hg}$ . About 2% of the added  $^{203}\text{Hg}$  was recovered during the first week after flooding and the recovery decreased to about 1%

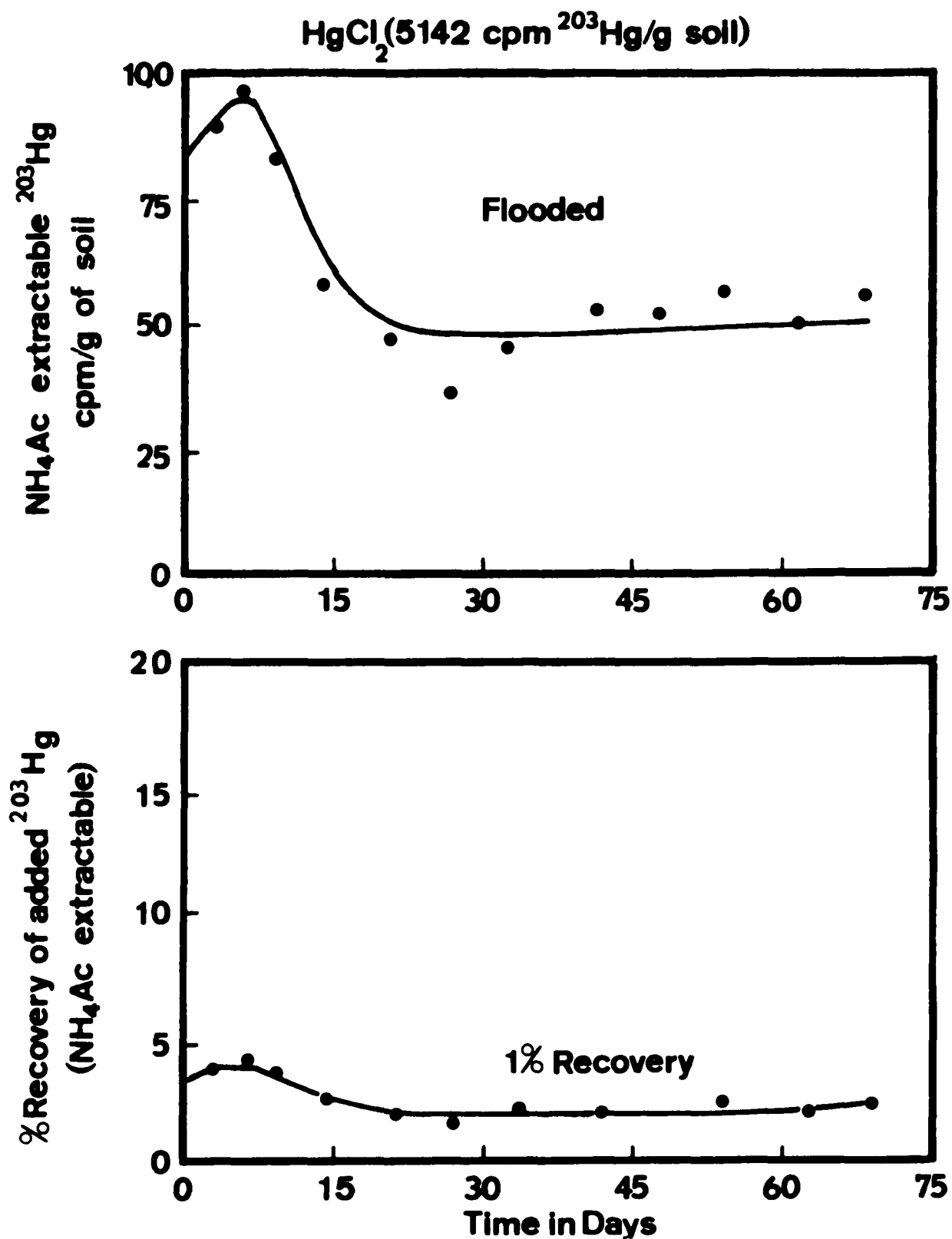


Figure 20. The effects of an anaerobic (flooded) soil system on the ammonium acetate extractable <sup>203</sup>Hg originating from <sup>203</sup>Hg labelled mercury chloride added to a Crowley silt loam.

on the onset of intense reducing conditions. This recovery figure was again one-third of that calculated for the  $^{35}\text{S}$  labelled mercury sulfide incubated in the flooded soil. However, the same percent of  $^{203}\text{Hg}$  was recovered from  $^{203}\text{HgCl}_2$  and  $^{203}\text{HgS}$  when mixed with an anaerobic soil. The recovery of mercury was not increased by the rigorous extraction procedure, even though a soluble form of mercury was added to the soil. These data suggest that the very soluble mercury compound readily disassociated and the mercury ion was quickly complexed as an insoluble salt or strongly adsorbed to the cation exchange complex of the soil. The subsequent decrease in ammonium acetate-extractable  $^{203}\text{Hg}$  after several days of incubation, further suggests that mercury may be complexed or precipitated by sulfides produced during intense reducing conditions. Extractable mercury decreased about 8 days after submergence. It has been previously shown that intense reducing conditions with respect to maximum sulfide accumulation occurred about 7 to 10 days after submergence.

A final mercury evaluation was made to study the movement of  $^{203}\text{HgCl}_2$  from the floodwater overlying an intensely reduced and a sterile Crowley silt loam. Prior to adding the mercury, the soil was flooded and incubated for 21 days to insure that intense reducing

conditions were prevalent. The soluble  $^{203}\text{Hg}$  was added to the floodwater overlying the soil and the floodwater radioactivity monitored regularly for 25 days. As a control series the  $^{203}\text{HgCl}_2$  was added to the floodwater of a Crowley soil treated similarly to the other. However, at the termination of the 21 day incubation period the soil was sterilized by steam and reincubated for two weeks prior to adding the radioactive mercury. The soil appeared to be sterile when compared to the soil that was not autoclaved. The radioactivity of the floodwater was assayed as in the nonsterile series. The results of this study are found in Figure 21. The floodwater  $^{203}\text{Hg}$  changes were given as cpm  $^{203}\text{Hg}$  in a 2 ml aliquot of the floodwater. The nonsterile, microbially active soil resulted in the greatest decrease in the mercury added to the floodwater, with 96% of the mercury moving out of the floodwater after 2 days. In the sterile series 80% of the floodwater mercury had disappeared after 5 days. The microbially active system where sulfide production was evident resulted in a 16% greater decrease in the floodwater mercury. These data suggest that the active microbial population play a significant role in mercury movement or precipitation as the insoluble sulfide in a flooded soil.

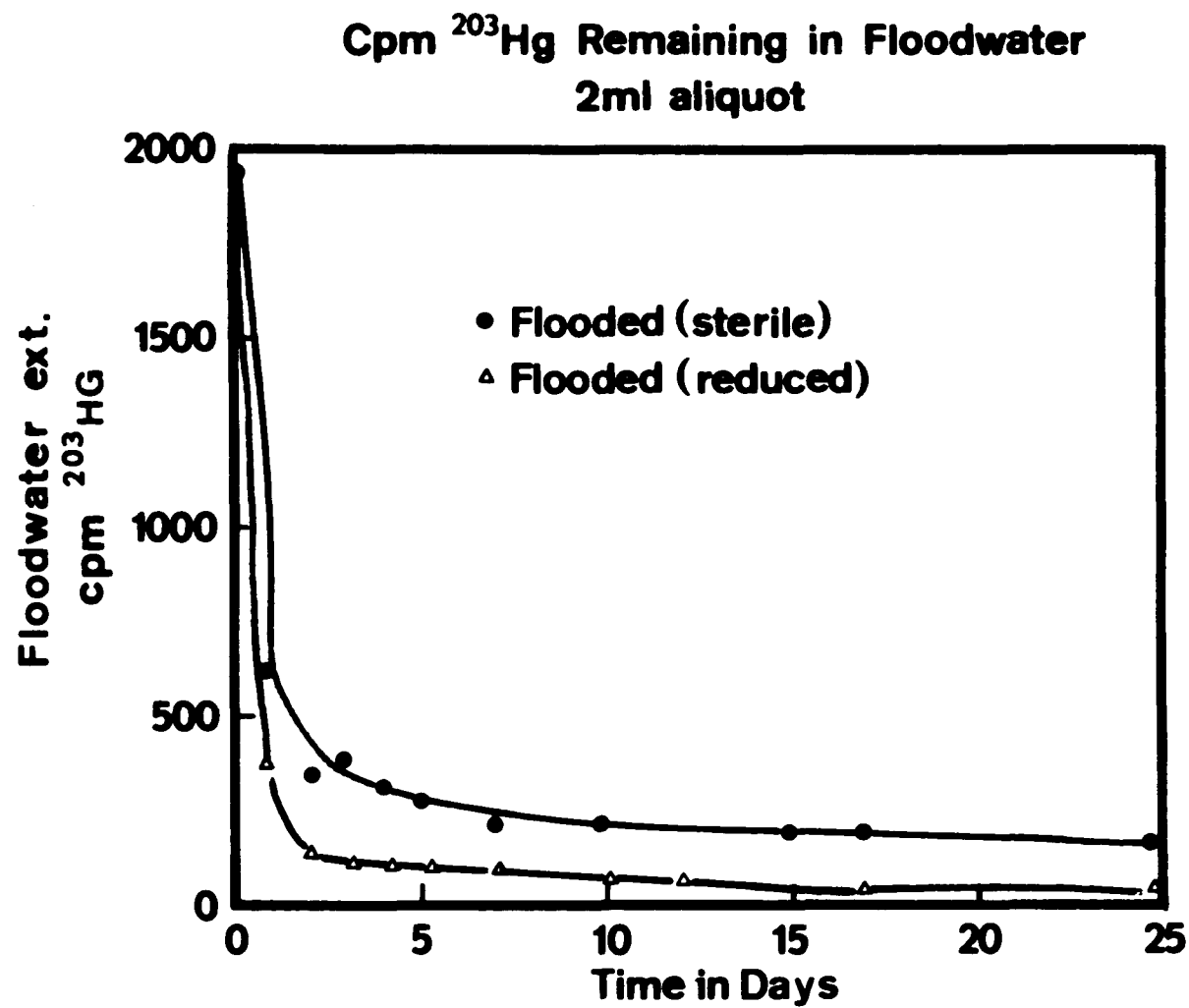


Figure 21. The movement of  $^{203}\text{Hg}$  labelled mercury chloride added to the floodwater of a sterile and nonsterile highly reduced flooded Crowley silt loam.

Mercury sulfide oxidation or decomposition as measured by an increase solubility of the mercury or sulfur is minimal in a flooded soil. Mercury sulfide appeared to be stable under flooded conditions although about 1% of the mercury appeared to dissociate from the salt and was recovered by a water extract. However, Fagerstrom and Jernelov (1970) showed that a small amount of highly soluble methylmercury was formed from pure mercuric sulfide in flooded organic sediments although the transformation was much slower than when mercuric chloride was the mercury source. Mercury mixed with the flooded soil appeared to be stable whether the source of mercury was the insoluble sulfide or soluble chloride because only about 1% of mercury was recovered from either source. However, in an aerobic soil mercury sulfide appeared to be slightly unstable as evidenced by a three-fold increase in recovery of added mercury. Consequently, when the aerobic soil was flooded the recovery of extractable mercury decreased to the same relative value determined for the soil that had been flooded continuously. Mercury that dissociated from the sulfide salt appeared to be strongly complexed or sorbed in the aerobic or anaerobic soil as evidenced by the lower percent recovery of added mercury as compared to sulfur recovered from mercury

sulfide. Mercury sulfide labelled with  $^{203}\text{Hg}$  or  $^{35}\text{S}$  was incubated under identical conditions. Further evidence of the capability of a soil to complex or sorb soluble mercury is shown by the high rate of movement of mercury (as  $\text{HgCl}_2$ ) from the floodwater overlying a sterile and nonsterile reduced soil. Also, little mercury could be recovered from  $\text{HgCl}_2$  added to a soil that was flooded and then extracted with normal ammonium acetate. This work is in agreement with that of the Geological Survey Staff (1970) where mercury was believed to be readily and strongly sorbed onto particulate matter suspended in water and bottom sediments. A very stable and highly insoluble salt of toxic mercury (mercuric sulfide) slowly decomposes into more soluble fractions in an aerobic soil and to a very small degree in an anaerobic soil. Mercury is also retained in large quantities by a strong complexing and sorption mechanism in flooded and nonflooded soils.

G. Effect of the Rice Plant on Metal Sulfides in a Submerged Soil

Rice (Oryza sativa), commonly grown on flooded soils where reduction processes are usually intense, has the capability of transporting oxygen into the soil adjacent to the root hairs and create relatively oxidized conditions in this zone (Alberda, 1953; Aomine,

1962; Armstrong, 1964, 1967; Mitsui, 1964; Ponnampetuma, 1964). In a flooded soil, micronutrient elements such as Fe, Mn, Zn and Cu readily form insoluble sulfide salts and presumably must be oxidized or separated from the sulfide prior to uptake and utilization by aquatic plants. Mercury, although not a micronutrient element, also readily forms sulfide salts in an anaerobic soil and this salt as well as other metal sulfides has previously been shown to be relatively stable under reducing conditions. However, the oxidation of the rooting zone by aquatic plants may render a portion of this stable mercury compound soluble and result in it being absorbed by the plant. A series of experiments was set up to evaluate the effect of the growing rice root on the oxidation of metal sulfides labelled with radioactive  $^{35}\text{S}$ . Subsequent plant uptake of the oxidized radioactive sulfur originating from the sulfide salt was measured. Plant uptake of  $^{35}\text{S}$  was used to evaluate the oxidative effect of the rice root because sulfide-sulfur must be oxidized before being taken up by the roots of a plant. For this evaluation, radioactively labelled metal sulfide salts were placed in the lower rooting zone of the rice plant growing in an intensely reduced flooded Crowley silt loam. Results of this study in which rice was grown on soil tagged with radioactively labelled metal sulfides ( $\text{MnS}$ ,  $\text{FeS}$ ,



ZnS, CuS and HgS) are shown in Table 1 and in Figure 22. Soluble sodium sulfide ( $\text{NaS}$ ;  $K_{sp} = 1 \times 10^{-1}$ ) labelled with  $^{35}\text{S}$  was used as a control treatment. To insure that there was no diffusion of atmospheric oxygen through the floodwater into the sulfide labelled soil a buffer zone of 80 mm of unlabelled soil was placed over the labelled soil. A study by Patrick and DeLaune (1971) demonstrated that soil oxidizing conditions with respect to sulfide oxidation progressed to a maximum depth of 12 mm after 18 weeks of incubation.

The data in Figure 22 are presented with the solubility product constants of the sulfide salts displayed on the horizontal axis and with the relative percent uptake of added  $^{35}\text{S}$  by the rice plant shown on the vertical axis. The mercuric sulfide treatment had rice grown on it for 42 days, the other sulfide treatments had rice grown on them for 21 days. This was done because of poor initial growth on the mercuric sulfide treated soil. The poor growth could have been due to a small amount of very toxic ionic mercury contamination of the insoluble sulfide salt.

The sodium sulfide treatment mixed into the lower rooting zone of the rice plant resulted in an uptake of 4.49% of the added  $^{35}\text{S}$  from the sulfide salt. This very soluble and easily oxidizable sulfide salt resulted in the greatest uptake of  $^{35}\text{S}$ . These data

Table 1. The effects of  $^{35}\text{S}$  labelled  $\text{Na}_2\text{S}$ ,  $\text{MnS}$ ,  $\text{FeS}$ ,  $\text{ZnS}$ ,  $\text{CuS}$ , and  $\text{HgS}$  placed in the lower rooting zone of a Crowley silt loam, on the yield of the aerial portion of the rice plant, uptake of  $^{35}\text{S}$  by the rice plant, and percent uptake of the added  $^{35}\text{S}$  by the rice plant.

Treatment	Ksp	Total added $^{35}\text{S}$ ---cpm---	Yield grams	Uptake		Percent uptake of added $^{35}\text{S}$
				Total -cpm-	Per 0.1g plant material -----cpm-----	
$\text{Na}_2^{35}\text{S}$	$1.0 \times 10^{-1}$	876,053	0.158	39,422	24,950	4.49
$\text{Mn}^{35}\text{S}$	$1.4 \times 10^{-15}$	1,008,000	0.326	6,315	1,774	0.62
$\text{Fe}^{35}\text{S}$	$3.7 \times 10^{-19}$	960,000	0.303	4,141	1,366	0.43
$\text{Zn}^{35}\text{S}$	$1.2 \times 10^{-23}$	1,026,900	0.313	3,948	1,261	0.38
$\text{Cu}^{35}\text{S}$	$3.5 \times 10^{-38}$	724,875	0.204	1,287	631	0.18
$\text{Hg}^{35}\text{S}$ <u>1/</u>	$1.0 \times 10^{-50}$	1,410,900	0.174	0	0	0

1/Rice was grown on the  $\text{HgS}$  treated soil for 42 days as compared to 21 days for the other sulfide treatments.

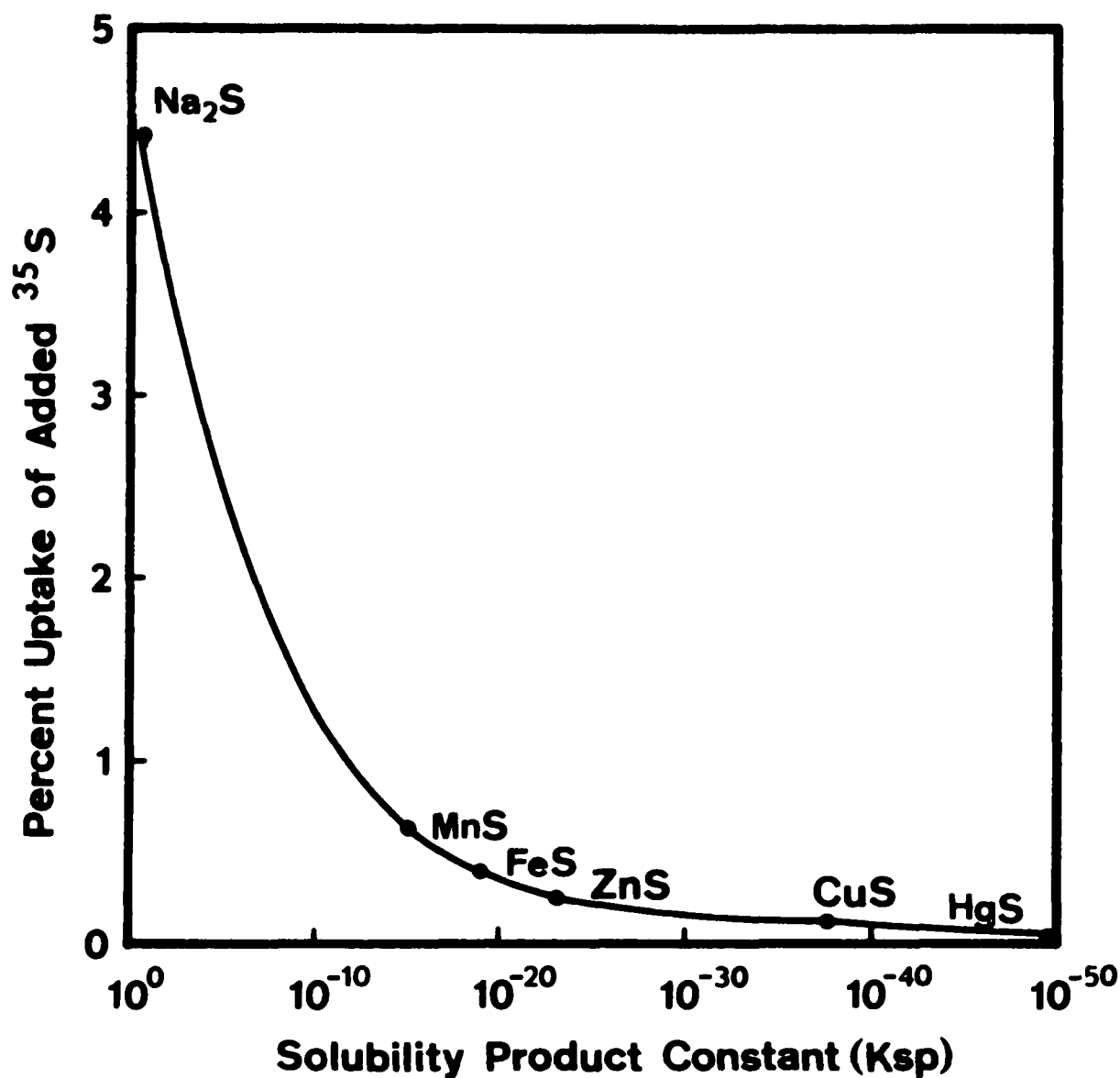


Figure 22. The percent uptake by the rice plant of <sup>35</sup>S from <sup>35</sup>S labelled Na<sub>2</sub>S, MnS, FeS, ZnS, CuS, and HgS placed in the lower rooting zone of a Crowley silt loam as related to the solubility product constant of the respective sulfide salt.

clearly demonstrate the capacity of the rice root to create a zone of soil where oxidizing conditions prevail and toxic sulfide may be oxidized chemically or microbially to the higher oxides of sulfur and subsequently taken up by the plant roots.

The more insoluble manganous sulfide treatment as compared to the sodium sulfide treatment resulted in an almost seven-fold less uptake of  $^{35}\text{S}$ . Manganous sulfide is at least 1400 times more insoluble than  $\text{Na}_2\text{S}$  and consequently could be more stable in the oxidized zone of the rice root. However, it was observed that the rice plant had presumably caused the oxidation and subsequent uptake of about 0.62% of the added  $^{35}\text{S}$ .

Ferrous sulfide added to the Crowley soil resulted in about 0.43% uptake of the added  $^{35}\text{S}$  by the rice plant. However, less  $^{35}\text{S}$  was taken up by the rice plants growing in the ferrous sulfide treated soil than by those growing in the manganous sulfide treated soil. The oxidized iron system immediately adjacent to the rice root could be visually distinguished by a red zone (forms of iron oxide) around the root hair as contrasted to the dark grey soil a few millimeters from the root.

Zinc, which exists in micro-amounts in most rice soils, can easily be rendered unavailable to the rice plant by precipitation as the insoluble sulfide. Consequently, an imbalance in the nutrition of the rice

plant may be created in soils that are particularly low in zinc. The data from this study indicate that the rice plant utilized 0.38% of the added  $^{35}\text{S}$  from the zinc sulfide. Again, the relative percent uptake was less from  $\text{ZnS}$  than from the previous more soluble sulfide salt.

The copper sulfide treatment resulted in about 0.18% uptake of the added radioactive sulfur from the sulfide salt. The oxidative capacity of the rice plant was less effective in decomposing insoluble copper sulfide to a more soluble form for plant uptake of the  $^{35}\text{S}$ . Consequently, the breakdown of copper sulfide would be very important with respect to the copper nutrition of the aquatic plant.

The rice plant did not take up any  $^{35}\text{S}$  from the mercury sulfide treated soil. Mercury sulfide, the most insoluble of the sulfides did not appear to be affected by the oxidative capacity of the rice roots. This effect may also have been caused by the toxic nature of the mercury itself, which was further demonstrated by the poor initial growth of the rice on the mercury sulfide treated soil. Consequently, the rice had to be grown for a longer period of time to insure a good root proliferation in the labelled soil. Mercury sulfide, an insoluble salt which may occur when soluble mercury is placed in an anaerobic soil

environment appears to be relatively stable in the oxidized zone of a rice root.

Standard free energies of formation of MnS, FeS, ZnS, CuS and HgS are all negative (Garrels and Christ, 1965), which suggests that the formation of the salts from their respective components is spontaneous. Therefore, in a typical soil solution containing Mn, Fe, Zn, and Cu and where anaerobic conditions (sulfide evolution) are intense, the metal sulfide formation may occur spontaneously. Flooded rice soils that contain iron and manganese usually contain zinc and copper in lesser amounts, and under intense reducing conditions the heavy metals may occur as the very insoluble and unavailable sulfide salts.

Armstrong (1964) measured the rate of diffusion of oxygen from the rice root and suggested that sulfide tolerance of some aquatic plants was due to oxidation of the sulfide by plant diffused oxygen. This is clearly evidenced by plant uptake of  $^{35}\text{S}$  from the labelled sodium sulfide as well as from the metal sulfides. The toxic nature of sulfide is well documented, and research by Okajima and Takagi (1955, 1956) show that very little sulfide passes into the rice root. They further reported that the very small amount that does move into the root remains there. The oxidation of the sulfide salts of Mn, Fe, Cu and Zn suggest that

the oxidative capacity of the rice root plays an important role in the micronutrient element nutrition of the rice plant. It is well documented that manganese and iron readily form sulfide salts in flooded rice fields and the previous data suggested that the rice roots rendered such insoluble salts to a more soluble and subsequently more available form for the rice plant. Aomine (1962) reported that the grey reduced soil of a rice paddy was mottled with red oxidized areas adjacent to the growing roots of the rice plant. This effect was noticed in the lower, very reduced layer of rice fields. Armstrong (1967) suggested that iron salts rendered more soluble by reduction may act as sinks for oxygen diffused through the plant roots. This theory was supported by the thick covering of hydrated ferric oxide that he found around the roots of rice plants growing in reduced soils. Zinc and copper are also required nutrient elements for the rice plant and the sulfide salts of these metals are even more insoluble. The data from the present study suggest that the rice root was capable of creating a condition where the zinc and copper sulfides were oxidized into more soluble and available forms. These facts point out the importance of the oxidative capacity of the rice plant in its micronutrient element utilization. The data in Figure 22 show that the uptake of

$^{35}\text{S}$  from the radioactively labelled metal sulfide salts appeared to be directly related to the solubility of the salt. Of more importance, the data suggest that the oxidative capacity of the rice root plays a significant role in the micronutrient element nutrition of the plant. The data also suggest that  $\text{HgS}$  was stable in the oxidized zone around the rice root for the growth time encompassed by this study. However, it was previously shown in a completely aerobic soil that mercury sulfide slowly changed into more soluble fractions. The entire soil sample of the previous heavy metal sulfide studies were aerobic, whereas, the rice root was only in contact with a small fraction of the total soil. Also, the toxicity of mercury may have had an inhibitive effect on the uptake of the sulfur by the rice plant. On the other hand, the relative stability of the mercury sulfide in the oxidized zone of the rice root suggests that the toxic mercury is unavailable for further contamination of the biological food chain.



## SUMMARY AND CONCLUSIONS

Studies were made of sulfate and sulfide transformations in a flooded and nonflooded Crowley silt loam. The rate of sulfide produced from an organic sulfur source (ground rice straw labelled with radioactive  $^{35}\text{S}$ ) was compared to the rate of sulfide evolved from an inorganic sulfur source ( $\text{Na}_2\text{SO}_4$  labelled with  $^{35}\text{S}$ ). It was found that about 18% of the added organic  $^{35}\text{S}$  was recovered by the end of a 50-day incubation period. A small amount of sulfide was still being produced from the organic source by the end of the incubation period. In contrast, about 90% of the added inorganic  $^{35}\text{S}$  was recovered after about 11 days of incubation. The rate of sulfide produced from the inorganic source had reached a peak after 11 days. The measurement of the rate of sulfide formation by use of a radioactive tracer was found to compare very favorably with the standard iodometric method for total sulfide analysis.

Sulfide formation from several inorganic sources of sulfur ( $\text{MnSO}_4$ ,  $\text{FeSO}_4$ ,  $\text{CuSO}_4$ ,  $\text{AgSO}_4$ ,  $\text{BaSO}_4$ ,  $\text{CaSO}_4$ ,  $\text{PbSO}_4$ ,  $\text{MgSO}_4$ ,  $\text{Na}_2\text{SO}_4$ , and elemental sulfur) added to a flooded Crowley silt loam was studied. Copper sulfate and silver sulfate at the rates used created

toxic conditions and no sulfide was produced. Barium sulfate, the most insoluble sulfate used, resulted in the lowest sulfide production. Only a few ppm sulfide were produced from this source. About 28% of the added elemental sulfur was recovered as sulfide. Reduction of other sulfur sources resulted in 65 to 80% recovery of the added sulfur as sulfide. For these last mentioned sulfur sources, the sulfide production did not appear to be related to the respective solubility products.

A study was initiated in which oxygen, ferric oxide, potassium nitrate, manganese dioxide, ferricitrophosphate, and ferric phosphate were added with sulfate-sulfur to separate samples of Crowley soil to evaluate the oxidizing effects of these compounds on sulfide production. The potassium nitrate treatment retarded initial sulfide production for 17 days and the ferricitrophosphate retarded it for 12 days. The more insoluble compounds such as manganese dioxide, ferric phosphate, and ferric oxide had little effect on the initiation of sulfide production. Whereas, ferric phosphate and manganese dioxide were effective in reducing the maximum rate of sulfide production by 24.1 and 16.7 ppm sulfide per day, respectively. However, the soluble oxidants had no appreciable effect on the maximum rate of sulfide formation in the flooded soil. The

redox potential of each treated soil was buffered at a higher value than the nontreated soil. This buffering was indicative of the presence of an oxidized compound in the flooded soil. The oxygen treatment was slightly effective in delaying the initiation of sulfide formation and reducing the maximum rate of sulfide formation.

A study was conducted in which oxygen, potassium nitrate, manganese dioxide, ferricitrophosphate, and ferric phosphate were added to a flooded Crowley silt loam in which sulfide formation had reached a maximum. Generally, the 500 ppm oxygen treatment was most effective in oxidation of sulfide resulting in a two-thirds loss in sulfide. The higher concentration of potassium nitrate (1000 ppm  $O_2$  equivalent) resulted in complete oxidation of sulfide. The ferricitrophosphate treatment was less effective than  $KNO_3$  in the capacity to oxidize sulfides in the flooded soil. In contrast, ferric phosphate, which was less soluble than the previously mentioned oxidants, was effective in oxidizing the sulfide formed in the anaerobic soil. The manganese dioxide treatment was an ineffective sulfide oxidant when added to the anaerobic soil. The redox potential measurements of the reduced soils were increased almost immediately following the additions of oxygen, potassium nitrate, ferricitrophosphate and ferric phosphate. This was indicative of the presence of an oxidized compound.

A series of experiments were initiated to measure the extent of oxidation of several heavy metal sulfides incubated under continuously flooded and moist conditions (1/3 bar moisture tension). The heavy metal sulfides evaluated were manganous sulfide ( $K_{sp} = 1.4 \times 10^{-15}$ ), ferrous sulfide ( $K_{sp} = 3.7 \times 10^{-19}$ ), zinc sulfide ( $K_{sp} = 1.2 \times 10^{-23}$ ), copper sulfide ( $K_{sp} = 3.5 \times 10^{-38}$ ), and mercuric sulfide ( $K_{sp} = 1 \times 10^{-50}$ ). Each metal sulfide was labelled with  $^{35}\text{S}$ . The oxidation of the sulfide salts was evaluated by measuring the changes in the concentration of the water-extractable  $^{35}\text{S}$  originating from the insoluble salts during the 70-day incubation period. In the moist soil, the large soil pores were open thus allowing gaseous exchange and an oxidized soil system. The continuously flooded soil remained anaerobic. In the anaerobic soil, all of the metal sulfide salts appeared to be relatively stable as no increase in water-extractable  $^{35}\text{S}$  was noted. In fact, a decrease in solubility of the sulfide compounds under anaerobic conditions was indicated by a decrease in water-extractable  $^{35}\text{S}$ . In contrast, the aerobic soil incubation resulted in the release of significant amounts of water-soluble  $^{35}\text{S}$  from the sulfide salts. The concentration of water-soluble  $^{35}\text{S}$  extracted from the aerobic soils appeared to be only partially related to the solubility product of

the metal sulfide salt. About 45% of the added  $^{35}\text{S}$  in  $\text{MnS}$  was recovered as water-extractable sulfur, while 50% of the added  $^{35}\text{S}$  in  $\text{FeS}$  was recovered in the water extract. About 20% of the added  $^{35}\text{S}$  originating from  $\text{ZnS}$  was recovered from the water extract. Copper sulfide released about 25% of the added  $^{35}\text{S}$  as water-soluble sulfur. Even the very insoluble and relatively stable  $\text{HgS}$  released about 7% of the added  $^{35}\text{S}$  when incubated under aerobic conditions.

Additional investigations of mercury transformations in aerobic and anaerobic soils were carried out. An experiment was conducted in which  $\text{HgS}$  was synthesized and labelled with  $^{203}\text{Hg}$  in order to study the dissolution of this compound in a continuously flooded soil conditions and in a soil alternately moist and flooded. The  $\text{HgS}$  incubated in a continuously flooded soil was relatively stable with recovery of about 1% of the added  $^{203}\text{Hg}$  as water-soluble mercury. In contrast,  $^{203}\text{Hg}$  labelled  $\text{HgS}$  was added to a soil that was cycled from an aerobic to an anaerobic state. About 3% of the added  $^{203}\text{Hg}$  was recovered from the moist (aerobic) incubated soil and when flooded, the recovery of water-extractable mercury decreased to about 1%. These data suggest that small amounts of very toxic mercury were recycled into the aerobic soil environment even though originating from very insoluble and stable mercuric

sulfide. An experiment utilizing  $^{203}\text{Hg}$  was initiated in which soluble  $\text{HgCl}_2$  labelled with radioactive mercury was added to a soil that was subsequently flooded with water and incubated for 70 days. An ammonium acetate extraction of the intensely reduced flooded soil resulted in about 1% recovery of the added  $^{203}\text{Hg}$ . Extraction of the soil prior to the onset of intense reducing conditions resulted in only a slight increase in recovery of added  $^{203}\text{Hg}$ . Consequently, the mercury that dissociated due to the decomposition of mercury sulfide and the easily dissociated mercury added as  $\text{HgCl}_2$  appeared to be readily and strongly complexed or adsorbed by the soil cation exchange mechanism. Similarly,  $\text{HgCl}_2$  labelled with  $^{203}\text{Hg}$  was added to the floodwater of a highly reduced soil and to the floodwater of a reduced soil that had been sterilized. Approximately 96% of the  $^{203}\text{Hg}$  moved out of the floodwater of the nonsterile soil within 2 days of adding. In the sterile soil only 80% of the  $^{203}\text{Hg}$  had moved out of the floodwater after 5 days. These data suggest that the active microbial population has a significant role in the movement of mercury from the floodwater to the sediment.

The oxidative effect of the growing rice plant on heavy metal sulfides placed in the lower rooting zone of a flooded soil was evaluated. The heavy metal

sulfides;  $\text{MnS}$ ,  $\text{FeS}$ ,  $\text{ZnS}$ ,  $\text{CuS}$  and  $\text{HgS}$ , were labelled with  $^{35}\text{S}$  and the percent uptake of the added  $^{35}\text{S}$  by the rice plant was used as an indication of the ability of the rice roots to oxidize sulfide to sulfate and absorb the sulfate. A control series with  $^{35}\text{S}$  labelled soluble  $\text{Na}_2\text{S}$  was used. The data demonstrated that plant uptake of  $^{35}\text{S}$  from sulfide salts was related to the solubility of the respective salt. The rice plant utilized about 4.49, 0.62, 0.43, 0.38, 0.18 and 0% of the added  $^{35}\text{S}$  from  $\text{Na}_2\text{S}$ ,  $\text{MnS}$ ,  $\text{FeS}$ ,  $\text{ZnS}$ ,  $\text{CuS}$ , and  $\text{HgS}$ , respectively. The sulfide salts are listed in decreasing order of their solubility. These data suggest that the rice plant was capable of oxidizing insoluble and relatively stable metal sulfides present in an anaerobic environment and utilizing the oxidized sulfur in its nutrition. The metal bonded to the sulfide might also be taken up by the plant or recycled as a more active soil or sediment constituent. The rice plant did not appear to oxidize any of the mercury sulfide as no  $^{35}\text{S}$  was taken up by the plant.

On the basis of the results obtained the following conclusions were drawn:

1. Sulfide was produced from organic material commonly plowed into a rice soil; the rate of sulfide production was relatively slow compared to the rate at which sulfide was produced from sulfate.

2. Sulfide production from  $\text{MnSO}_4$ ,  $\text{FeSO}_4$ ,  $\text{CaSO}_4$ ,  $\text{PbSO}_4$ ,  $\text{MgSO}_4$ , and  $\text{Na}_2\text{SO}_4$  placed in a flooded soil was significant with 65 to 80% recovery of the added sulfur. Sulfide formation did not appear to be related to the solubility products of the sulfur source. Elemental sulfur was an intermediate source for sulfide formation with about one-third of the added elemental sulfur being recovered as sulfide. Only a few parts per million of sulfide were produced from the very insoluble  $\text{BaSO}_4$ .
3. Soluble oxidizing compounds (potassium nitrate and ferricitrophosphate) mixed with a soil prior to flooding were effective in delaying initial sulfide production. Insoluble oxidants (manganese dioxide and ferric phosphate) were effective in reducing the maximum rate of sulfide formation.
4. Oxygen, potassium nitrate, ferricitrophosphate, and ferric phosphate mixed with a flooded soil after sulfide content reached a maximum were effective in reducing the amount of accumulated sulfide.  $\text{MnO}_2$  was ineffective when applied after flooding.
5.  $\text{MnS}$ ,  $\text{FeS}$ ,  $\text{ZnS}$ ,  $\text{CuS}$  and  $\text{HgS}$  were found to be stable in flooded soils when evaluated by



measuring the release of sulfur. The metal sulfide salts were relatively unstable in aerobic (1/3 bar moisture tension) soils and the rates of oxidation of the sulfides were partially related to their respective solubility product constants.

6. Soluble mercury mixed with a flooded soil or added to the overlying floodwater was readily and strongly complexed or adsorbed on the soil cation exchange complex.
7.  $\text{MnS}$ ,  $\text{FeS}$ ,  $\text{ZnS}$ , and  $\text{CuS}$  placed in an anaerobic, intensely reduced soil were apparently partially oxidized by the growing roots of rice plants and the sulfur subsequently taken up by the plants. The rates of oxidation of the sulfide salts and uptake of sulfur were directly related to the solubility product of the sulfides. Mercury sulfide did not appear to be oxidized by the rice roots since no sulfur was absorbed from this source.

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## VITA

Robert McLure Engler was born October 18, 1941, Alexandria, Rapides Parish, Louisiana. He was graduated from Bolton High School, Alexandria, in 1959.

He attended McNeese State College, Lake Charles, Louisiana for two semesters prior to serving a two and one-half year tour of active duty in the U. S. Navy. He was honorably discharged from the U. S. Navy in August, 1963.

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## EXAMINATION AND THESIS REPORT

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Major Field: Agronomy

Title of Thesis: Oxidation-Reduction Reactions of Sulfate and Sulfide  
in Flooded and Nonflooded Soil

Approved:

Wm. H. Patrick Jr.  
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Date of Examination:

June 30, 1972